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RIVM Briefrapport 2016-0036
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Een kwart van de gebruikers van e-sigaretten zijn op dit product overgestapt om omstanders te ontzien (meeroken). Toch worden ook bij het gebruik van e-sigaretten schadelijke stoffen uitgeademd, zoals propyleenglycol, nicotine en nitrosamines. De hoeveelheid die wordt uitgeademd is sterk afhankelijk van de samenstelling van de gebruikte vloeistof, de intensiteit van het dampen (frequentie en inhalatie), en de ventilatie en afmetingen van de ruimte waarin wordt gedampt. Dit bepaalt ook in hoeverre gezondheidsrisico’s kunnen optreden. Dit blijkt uit onderzoek van het RIVM dat is uitgevoerd in opdracht van het ministerie van VWS.

Een deel van de schadelijke stoffen blijft achter in de 'damper' nadat ze zijn ingeademd. Doordat een e-sigaret alleen wordt geactiveerd als de gebruiker een trekje neemt, smeulen e-sigaretten niet door. Daardoor komen er geen schadelijke stoffen vrij als er geen trekje wordt genomen. Bij tabakssigaretten is dat wel het geval. Afhankelijk van bovenstaande factoren kunnen omstanders lichte gevoelens van keel-, neus- en oogirritatie ervaren. Veel dampers (78 procent) gebruiken vloeistoffen die nicotine bevatten. Hierdoor kunnen omstanders gezondheidseffecten ondervinden als hartkloppingen en een verhoogde bloeddruk. Bij gebruik van nicotinevrije vloeistoffen ontstaan deze effecten dus niet.

Voor dit onderzoek heeft het RIVM de chemische samenstelling gemeten van de damp die e-sigaretgebruikers uitblazen. Hierbij is uitsluitend gekeken naar de toxicologische gezondheidsrisico’s. De conclusies zijn gebaseerd op de meest recente kennis over de risico’s van stoffen wanneer ze worden geïnhaleerd. Van sommige stoffen is echter nog niet bekend in hoeverre zij een risico voor de gezondheid vormen. Daarom wordt aanbevolen om de gezondheidseffecten van e-sigaretten voor gebruikers en omstanders nauwgezet te blijven volgen.

Kernwoorden: e-sigaret, omstanders, meedampen, blootstelling, risicobeoordeling, gezondheidseffecten

Synopsis

The health risks of e-cigarettes to bystanders

One fourth of e-cigarette users started using this product to reduce exposure of bystanders (passive smoking). However, e-cigarette users also exhale harmful compounds, including propylene glycol, nicotine and nitrosamines. The composition of the e-liquid, the vaping intensity (frequency and inhalation), and the ventilation and dimensions of the room in which e-cigarettes are being used have a large bearing on the amounts of harmful compounds that are exhaled and, consequently, on the extent to which health risks can occur. The Dutch National Institute for Public Health and the Environment (RIVM) conducted research on the health risks of e-cigarette use to bystanders, on behalf of the ministry of Health, Welfare and Sport (VWS)

Harmful components are partially retained by ‘vapers’ after inhalation. Because e-cigarettes are only active when users take a puff, e-cigarettes do not continue to smolder between puffs. Therefore, e-cigarettes do not emit harmful compounds when no puff is being taken, in contrast to tobacco cigarettes. Depending on the conditions, bystanders may experience mild irritation of the throat, nose and eyes. Many vapers (78 percent) use nicotine-containing e-liquids. As a result, bystanders may experience palpitations and increased blood pressure. These effects do not occur when nicotine-free liquids are used.

For this investigation, the RIVM measured the chemical composition of the breath exhaled by e-cigarette users. Only toxicological health risks have been considered. The conclusions are based on the most recent insights regarding the health risks of compounds upon inhalation. For some compounds, insufficient data is available to establish whether they present risks to human health. We therefore recommend to continue to monitor closely new developments regarding the health effects of e-cigarettes to bystanders and users.

Keywords: e-cigarette, bystanders, passive vaping, exposure, risk assessment, health effects

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 beoordeling gemaakt van de toxicologische risico's voor omstanders. Er is niet gekeken naar andere mogelijke gezondheidseffecten, zoals schade ontstaan door inslikken van e-vloeistof door kinderen, het ontploffen van een batterij of mogelijke effecten op populatie niveau doordat men roken weer normaler gaat vinden.

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 ontstaat in de tijd dat er van een sigaret geen trekje wordt genomen, de zogenaamde zijstroomrook ('sidestream smoke'). De rest van de schadelijke stoffen wordt uitgeademd door de roker. Bij e-sigaretten worden omstanders uitsluitend blootgesteld aan aerosol die eerst door de gebruiker is geïnhaleerd en vervolgens uitgeademd.

Voor het onderzoek is experimenteel bepaald wat de samenstelling is van de adem die ervaren e-sigaret gebruikers uitademen. Daarbij zijn de hoeveelheden van stoffen gemeten waarvan in eerder onderzoek is vastgesteld dat ze tot gezondheidseffecten voor de gebruikers zelf kunnen leiden. Het gaat om nicotine, propyleenglycol, glycerol, aldehydes, tabakspecifieke nitrosamines (TSNAs) 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 de duur van, het interval tussen en het volume van trekjes zijn in overeenstemming met gepubliceerde gegevens van andere onderzoekers.

Uit metingen van de samenstelling van de adem die ervaren e-sigaret gebruikers uitademen tijdens het gebruik van e-sigaretten blijkt dat een deel van de schadelijke stoffen uit de geïnhaleerde e-sigaret aerosol achter blijft in de gebruikers. Na uitademing zullen de uitgeademde stoffen 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.

De risicobeoordeling is uitgevoerd voor twee scenario's:

1) een dagelijkse autorit waarbij een kind wordt blootgesteld aan stoffen uitgeademd door twee e-sigaretgebruikers

2) blootstelling van een volwassen persoon in een kantoorruimte aan stoffen uitgeademd door één e-sigaretgebruiker gedurende een halve werkdag.

De blootstelling van de omstanders kan zo gerelateerd worden aan het ontstaan van schadelijke effecten op de gezondheid. Voor het eerste scenario (auto) kan geconcludeerd worden dat bij een nicotine-houdende e-sigaret blootstelling aan nicotine kan resulteren in een

verhoogde hartfrequentie en verhoogde systolische bloeddruk (vergelijkbaar met de verhoging in bloeddruk die te verwachten is van de inname van de hoeveelheid cafeïne uit twee of drie koppen koffie). Dit is uiteraard niet het geval bij gebruik van nicotine-vrije e-vloeistoffen. Als gevolg van blootstelling aan propyleenglycol (component van de dragervloeistof) kan niet uitgesloten worden dat een milde irritatie van neus, keel en ogen kan optreden. Specifiek bij e-vloeistoffen die relatief hoge concentraties 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 kanttekeningen worden geplaatst. De meeste e-vloeistoffen bevatten geen, of slechts lage concentraties TSNAs. Ze zijn soms aanwezig als verontreiniging in de door fabrikanten gebruikte ingrediënten voor e-vloeistoffen.
Voor het tweede scenario (kantoor) kunnen bovengenoemde effecten van nicotine voor omstanders niet worden uitgesloten, maar er worden in dat geval geen effecten van propyleenglycol verwacht. Met betrekking tot de TSNAs is het niet mogelijk om duidelijke conclusies te trekken voor dit scenario. De beoordeling van dit scenario zou kunnen leiden tot de conclusie dat het tumorrisico zeer laag is, maar dit kan niet met voldoende zekerheid worden vastgesteld.
De risico's zijn sterk afhankelijk van het gedrag van de e-sigaret gebruiker, de afmetingen en ventilatie van de ruimte waarin gedampt wordt en de gebruikte e-vloeistoffen. 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.

Definities en afkortingen

PG	Propyleenglycol
TSNAs	tabakspecifieke nitrosamines. Dit is een verzamelterm voor vier stoffen: N-nitrosonornicotine (NNN), N'-nitrosoanatabine (NAT), N-nitrosoanabasine (NAB) en 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
aerosol	In het kader van dit rapport wordt hiermee de damp aangeduid die door een e-sigaret wordt gegenereerd en vervolgens door gebruikers wordt geïnhaleerd.
Uitgeblazen adem	In het kader van dit rapport wordt hiermee de adem bedoeld die e-sigaret gebruikers uitblazen als ze een e-sigaret gebruiken.

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 uitademen door de gebruiker en verspreiding 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 adem damp en is geen rekening gehouden met mogelijke andere effecten, zoals mogelijke effecten op populatie niveau doordat men roken weer normaal gaat vinden (3, 4), verwondingen ontstaan door het exploderen van e-sigaretten (5-7) of vergiftigingen door het inslikken van nicotine-houdende e-vloeistof door kinderen (8-11).

Indeling van dit briefrapport
Voor aanvullende details van de gebruikte methoden en resultaten is een technische appendix opgenomen vanaf hoofdstuk 7. Deze is geschreven in het Engels om het voor een internationaal publiek toegankelijk te maken. Daarbij zijn delen van de Nederlandse tekst herhaald ten behoeve van de leesbaarheid.

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 (12).

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" (1), 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 ruimten 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, en sommige staten en steden van de USA. Daarbij zijn eventuele toxicologische gezondheidseffecten niet altijd de belangrijkste overweging geweest. Zo is in Frankrijk besloten (13) 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: e-sigaret aerosol en uitgeblazen adem

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 (*'aerosol'*) en de adem die door de gebruikers vervolgens wordt uitgeblazen en die zich verspreidt in de omgeving (*'uitgeblazen adem'*). Uit verschillende studies is gebleken dat er grote verschillen zijn in de samenstelling van uitgeblazen adem bij e-sigaret gebruik en de door e-sigaretten geproduceerde aerosol (14-16). Dat is niet verrassend, omdat een deel van de stoffen in e-sigaret aerosol worden gedeponeerd of geabsorbeerd in de luchtwegen van de e-sigaret gebruiker, en niet meer worden uitgeademd. De mate waarin dit gebeurt, verschilt per stof en is afhankelijk van het dampgedrag van de gebruiker. In sommige gepubliceerde onderzoeken naar de schadelijkheid van e-sigaretten voor omstanders wordt gebruik gemaakt van aerosol die met behulp van een rookmachine wordt gegenereerd. Daarbij zullen echter de blootstelling en daaruit voortvloeiende gezondheidsrisico's overschat worden.

1.3 Definities: main-stream smoke en sidestream smoke

Gewone tabakssigaretten produceren ook rook als geen trekje wordt genomen omdat ze in de tussentijd doorsmeulen. De rook die daarbij ontstaat wordt *sidestream 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 (17).
E-sigaretten produceren geen equivalent van side stream smoke ("side stream aerosol"). Alleen als de gebruiker een trekje neemt wordt het verwarmingselement geactiveerd, en omstanders worden dus uitsluitend blootgesteld aan stoffen aanwezig in de uitgeblazen adem. In sommige studies is geen rekening gehouden met dit verschil, wat tot een overschatting leidt van de blootstelling van omstanders aan 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 met behulp van een rookmachine gegenereerde (verdunde) e-sigaret aerosol terwijl de samenstelling van de uitgeblazen adem 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 door e-sigaret gebruikers uitgeblazen adem. Fernandez *et al.* (15) hebben 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 bepaald. Omdat de studies echter op belangrijke punten verschillen zoals het aantal proefpersonen, hun dampgedrag, de duur van het experiment, het volume van de testkamer en de mate van ventilatie is het niet goed mogelijk om de resultaten onderling te vergelijken. Wel worden in de meeste studies meetbare hoeveelheden propyleenglycol en nicotine gevonden.
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, en dampgedrag van proefpersonen, etc.

1.5 Opzet van het huidige onderzoek

E-sigaretten worden vrijwel overal gebruikt (1). Om een risicobeoordeling te kunnen uitvoeren van verschillende scenario's (bijvoorbeeld een auto of een kantoorruimte) is besloten tot de volgende opzet. Allereerst is experimenteel vastgesteld wat het dampgedrag is bij normaal e-sigaretgebruik. Vervolgens is bepaald welke hoeveelheden van verschillende stoffen gebruikers uitblazen in de ruimte.
Aan de hand van deze meetgegevens is een risicobeoordeling uitgevoerd waarbij gekeken is naar de mogelijke gezondheidsrisico's voor omstanders in twee van tevoren gedefinieerde scenario's van e-sigaret gebruik. Het eerste 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 in een kantoorruimte aan de stoffen die worden uitgeademd door een e-sigaretgebruiker gedurende een deel van een werkdag.

2 Experimentele bepaling van de topografie van e-sigaret gebruik

Naar de topografie van het roken van gewone tabakssigaretten, d.w.z. parameters zoals de duur van, het interval tussen en het volume van trekjes is al veel onderzoek verricht. Over de topografie van e-sigaretgebruik (‘dampgedrag’) is veel minder bekend. Wel is uit verschillende onderzoeken gebleken dat dampgedrag verschilt van rookgedrag (18-20). De duur van een trekje is bijvoorbeeld over het algemeen langer bij dampers. Een mogelijke verklaring is dat e-sigaretgebruikers hun gedrag aanpassen omdat de hoeveelheid nicotine in de aerosol anders is (21). Gebruikers kunnen door hun dampgedrag te veranderen toch dezelfde hoeveelheid nicotine opnemen als bij het roken van tabakssigaretten.
Om inzicht te krijgen in de topografie van e-sigaretgebruik hebben we daarom voor het huidige onderzoek metingen verricht bij 18 ervaren e-sigaretgebruikers (>3 maanden dagelijks gebruik). 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 (‘flowmeter’).

2.1 Methoden

2.1.1 Werving proefpersonen

Het onderzoek is beoordeeld en goedgekeurd door de Medisch Ethische Toetsingscommissie van 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-sigaretgebruik. Inclusiecriteria voor deelname aan de studie waren als volgt: 1) tussen de 18-55 jaar oud en 2) dagelijks gebruik van een e-sigaret met minimaal 6 mg/ml nicotine gedurende tenminste 3 maanden. Vrouwen die borstvoeding gaven, zwanger waren, of van plan waren dat te worden ten tijde van het onderzoek waren uitgesloten van deelname, evenals mensen die nadelige gezondheidseffecten ervaren hadden van hun e-sigaret gebruik.

2.1.2 Meting

Deelnemers mochten op de dag van het experiment vrij roken en/of e-sigaretten gebruiken voorafgaand aan het experiment om te vermijden dat ze een ongewone nicotine behoefte zouden ervaren. Deelnemers werden 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 aan gerelateerd zijn vermeden werden.

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). Hiervan gebruikten 485 personen (78%) een vloeistof met >6 mg/ml nicotine. 273 personen (44%) rookten 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.
Op basis van de in 2.1.1 genoemde criteria werden 18 personen geïncludeerd. Onder de 18 deelnemers aan het onderzoek waren 10 mannen (56%) en 8 vrouwen (44%). 40% procent van de geïncludeerde mannen en 62% van de geïncludeerde vrouwen waren *dual users*.

2.2.2 Topografie

Van 3 deelnemers werden door een defect aan de meetapparatuur geen meetwaarden verkregen. Er bleken aanzienlijke individuele verschillen te zijn in de topografie van de verschillende deelnemers en in het gedrag van een persoon gedurende het experiment. Dit is overeenstemming met resultaten andere onderzoeken. Robinson *et al.* hebben hier recentelijk een goed overzicht van gepubliceerd, aangevuld met hun nieuwe eigen metingen (20). Tabel 2.1 geeft een overzicht van de gevonden waarden voor de duur van, het interval tussen en het volume van trekjes.

Tabel 2.1: overzicht parameters damptopografie (n=15)

	percentielen		
	P5	**P95**	**mediaan**
puff duur (sec)	1,1	8,6	3,8
puff interval (sec)	6,6	121,0	43,0
puff volume (mL)	8,5	119,4	56,0

Ondanks de grote individuele verschillen zijn de mediaan van de duur (3.8 sec) en het volume (56 mL) in goede overeenstemming met de eerder door literatuuronderzoek (1) vastgestelde waarden (respectievelijk 4 sec en 55 mL).

3 Experimentele bepaling van de hoeveelheden schadelijke stoffen in uitgeblazen adem.

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

3.1 Methoden

Aan de proefpersonen werd gevraagd om een van tevoren vastgesteld aantal trekjes te nemen van een e-sigaret en na ieder trekje de eerste uitademing via een mondstuk uit te ademen op filters waaraan de relevante stoffen binden. Deze stoffen werden vervolgens ge-extraheerd van de filters en door chemische analyse werd de hoeveelheid van de verschillende stoffen bepaald. Omdat sommige stoffen ook van nature kunnen voorkomen in normale uitgeblazen adem of in de omgevingslucht werd aan deelnemers ook gevraagd om eerst gewoon uit te ademen op een filter (zonder het dampen van een e-sigaret). Deze controlefilters werden gelijktijdig geanalyseerd en gebruikt ter correctie van de achtergrond. Om op basis van de gemeten hoeveelheid in de uitgeblazen adem ook de concentratie te kunnen uitrekenen is ook het totale volume dat de deelnemers op de verschillende filters uitademden gemeten met een debietmeter (*flowmeter*).
In eerder onderzoek waren een aantal stoffen die aanwezig kunnen zijn in de aerosol van e-sigaretten geïdentificeerd die tot gezondheidsrisico's voor de gebruikers zelf kunnen leiden (1). Hierbij gaat het om nicotine, propyleenglycol, glycerol, aldehydes, tabakspecifieke nitrosamines (TSNAs) en metalen. Deze stoffen zijn daarom meegenomen in het huidige onderzoek. Van stoffen waarvan eerder was vastgesteld dat de concentraties in e-sigaret aerosol zodanig laag zijn dat blootstelling niet resulteert in significante gezondheidsrisico's voor de e-sigaretgebruiker, zoals vluchtige organische stoffen (VOCs), is geen analyse uitgevoerd, omdat de blootstelling van omstanders lager zal zijn dan van de e-sigaret gebruikers zelf.
De hoeveelheid van verschillende stoffen in de aerosol hangt uiteraard ook af van de gebruikte e-sigaret en vloeistof. Om een schatting te kunnen maken van de hoeveelheden die voor kunnen komen in de uitgeblazen adem 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-vloeistoffen 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 eerder uitgevoerde 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 nicotineconcentratie (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 vloeistoffen in het eerder uitgevoerde onderzoek (1).

3.2 Resultaten en discussie

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

Tabel 3.1: Overzicht van de gemeten stoffen in uitgeblazen adem. De 'hoeveelheid per eerste uitademing' is de gemiddelde hoeveelheid in de eerste uitademing na het nemen van een trekje. De concentratie is de gemiddelde* concentratie in die eerste uitademing na het nemen van een trekje. Voor het berekenen van de mediaan zijn alle data gebruikt, inclusief monsters waarvan de concentratie onder de kwantificatielimiet was. *) in totaal zijn 5 (nicotine/dragervloeistof) of 25 (aldehydes/metalen) 'eerste' uitademingen na het nemen van een trekje opgevangen op een filter.*

		hoeveelheid				**concentratie**			
		bereik				**bereik**			
	n	min	max	**mediaan**		min	max	**mediaan**	
nicotine	17	<LOQ	2140	108	ng	<LOQ	12391	323	ng / L
dragervloeistof									
propyleen glycol	17	<LOQ	127	<LOQ	µg	<LOQ	839	64	µg / L
glycerol	17	<LOQ	<LOQ	<LOQ	µg	<LOQ	<LOQ	<LOQ	µg / L
nitrosamines									
NNN	9	<LOQ	111	29	pg	<LOQ	961	84	pg / L
NAT	9	<LOQ	40	14	pg	<LOQ	172	47	pg / L
NAB	9	<LOQ	8	2	pg	<LOQ	16	9	pg / L
NNK	9	<LOQ	71	15	pg	<LOQ	403	39	pg / L
aldehydes									
formaldehyde	4	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
acetaldehyde	4	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
acroleine	4	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
metalen									
arseen	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
molybdeen	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
tin	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
cadmium	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
lood	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
zink	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
koper	3	<LOQ	2.92	<LOQ	ng	<LOQ	28	<LOQ	ng / L
nikkel	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
cobalt	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
mangaan	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
chroom	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
vanadium	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
uraan	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L

De hoeveelheden van deze stoffen in de eerste uitademing na het nemen van een trekje zijn zonder uitzondering lager dan die in de e-sigaret aerosol (1) die wordt ingeademd. Hiervoor zijn verschillende mogelijke oorzaken aan te wijzen. De belangrijkste oorzaak is dat een deel van de geïnhaleerde stoffen gedeponeerd of geabsorbeerd worden in de luchtwegen van de e-sigaretgebruiker. Daarnaast wordt in de eerste uitademing niet de volledige inhoud van de longen uitgeademd, en fractie van de stoffen in de longen kan later alsnog worden 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 waarin gekeken is naar de samenstelling van uitgeblazen adem bij e-sigaret gebruik zijn lage hoeveelheden nicotine en van de componenten van de dragervloeistof in de uitgeblazen damp gevonden (14, 16).
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.* (16), waaruit blijkt dat >99% van de nicotine wordt geabsorbeerd of gedeponeerd in de luchtwegen van gebruikers als deze 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 Beoordeling 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 uitgeblazen adem (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 uitgeblazen adem 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 (22). 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 (23), 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 uitgeblazen adem 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 uitgeblazen adem 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 (1) .

- *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 uitgeblazen adem 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 (1) .

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 uitgeblazen adem van 4 van de 17 vrijwilligers.

Op basis van de gemeten hoeveelheid in de uitgeblazen adem kan voor scenario 1 (auto) niet uitgesloten worden dat lokale effecten op de luchtwegen en ogen (irritatie van neus, keel en ogen) 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 uitgeblazen adem, *i.e.* glycerol was aanwezig in de uitgeblazen adem 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 uitgeblazen adem 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 uitgeblazen adem 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 uitgeblazen adem, *i.e.* deze aldehydes waren aanwezig in de uitgeblazen adem in een hoeveelheid lager dan de LOQ (na correctie van het achtergrondniveau). 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 uitgeblazen adem bij 8 van de negen vrijwilligers.
Op basis van de gemeten hoeveelheid in de uitgeblazen ademkan 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 uitgeblazen adem boven de LOQ bij 1 van de drie vrijwilligers. Op basis van de gemeten hoeveelheid in de uitgeblazen adem 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 uitgeblazen adem, *i.e.* deze stoffen waren aanwezig in de uitgeblazen adem 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 uitgeblazen adem 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 uitgeblazen adem van vrijwilligers die geselecteerde e-vloeistoffen 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 uitgeblazen adem 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 uitgeblazen adem 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 uitgeblazen adem werd gebruikt voor de risicobeoordeling. De metingen lieten een inter-individuele variatie zien in hoeveelheid stof aanwezig in de uitgeblazen adem. 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 uitgeblazen adem 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 irritatie van neus, keel en ogen 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 uitgeblazen adem 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-vloeistoffen is de hoeveelheid van een specifieke stof in de eerste uitademing afhankelijk van de geselecteerde e-vloeistoffen. Opgemerkt dient te worden dat alle, in de huidige studie, geselecteerde e-vloeistoffen nicotine bevatten. Nicotine zal vanzelfsprekend geen probleem vormen bij het dampen van nicotine-vrije e-vloeistoffen. Ook de aanwezigheid van tabaksspecifieke nitrosamines in de damp is sterk afhankelijk van het type e-vloeistof. Tabakspecifieke nitrosamines zijn gekoppeld aan de aanwezigheid van nicotine en/of tabaksextract in de e-vloeistof. Tabakspecifieke nitrosamines zullen geen probleem vormen bij het dampen van nicotine-vrije e-vloeistoffen 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 uitgeblazen

adem), welke inter- en intra-individuele variatie laat zien, zal van invloed zijn geweest op de gemeten hoeveelheid in de uitgeblazen adem en die is gebruikt in de risicobeoordeling.

5 Beperkingen

Voor de risicobeoordeling zijn twee scenarios gedefinieerd waarin sprake is van een relatief hoge blootstelling van omstanders. Hiertoe werd besloten omdat uit de metingen van de samenstelling van de uitgeblazen adem bleek dat een aanzienlijk deel van de geïnhaleerde propyleenglycol en nicotine (en waarschijnlijk ook andere stoffen) achterblijft in de luchtwegen van de gebruikers, en omdat de uitgeblazen stoffen sterk verdund raken in de omgevingslucht.

Kort samengevat betreft scenario 1 een dagelijkse rit met een auto waarin zich twee dampers bevinden en de omstander een kind is op de achterbank. Scenario 2 is een deel van een werkdag in een kantoor, waarin een volwassen omstander een kantoorruimte deelt met een dampende collega. Een meer gedetailleerde beschrijving van de scenario's is te vinden in 4.2.1. In deze scenario's was sprake van gezondheidsrisico's: voor het eerste scenario kan irritatie van de luchtwegen optreden als gevolg van de blootstelling aan propyleenglycol. Als een nicotine-houdende e-vloeistof wordt gebruikt, dan kunnen in beide scenario's hartkloppingen en een verhoging van de systolische bloeddruk optreden als gevolg van blootstelling aan nicotine. Voor vloeistoffen die een relatief hoge concentratie tabakspecifieke nitrosamines (TSNAs) bevatten kan een verhoogd risico op tumoren in de luchtwegen niet worden uitgesloten, maar daarbij moet worden opgemerkt dat de meeste e-vloeistoffen slechts lage concentraties TSNAs bevatten en dat nieuwe wetgeving het gebruik van ingrediënten die onnodig verontreinigd zijn met stoffen die schadelijk zijn voor de menselijke gezondheid (zoals TSNAs) niet toestaat (24) (zie ook paragraaf 5.6.1)

In dit hoofdstuk worden beperkingen van het onderzoek in rapport besproken die het gevolg zijn van (meet)onzekerheden, gemaakte aannames en de gekozen opzet.

5.1 Variatie in damptopografie en ademgedrag

Omdat omstanders uitsluitend worden blootgesteld aan stoffen die door de gebruikers worden uitgeblazen zijn de parameters die te maken hebben met inhalatie (het nemen van een trekje) en uitblazen van de adem van belang. Deze parameters omvatten het volume van een trekje, het interval tussen trekjes, het totaalvolume dat wordt geinhaleerd (diep of oppervlakkig), hoelang de adem wordt ingehouden na een trekje en het totaal uitgeblazen volume. Deze parameters kunnen de hoeveelheid stoffen die wordt uitgeblazen op verschillende manieren beïnvloeden. Systematisch onderzoek naar het effect van deze parameters valt buiten het bestek van dit onderzoek. Bovendien zijn onvoldoende gegevens beschikbaar betreffende normaal en uitzonderlijk dampgedrag. De vrijwilligers in het experiment mochten vrij dampen en ademen gedurende het experiment, en in de data die gebruikt is voor de risicobeoordeling is daarom ook sprake van spreiding tengevolge van individuele verschillen in damp- en ademgedrag. De waargenomen spreiding is vrij groot (zie hoofdstuk 8), wat in overeenstemming is met

gepubliceerde resultaten van anderen (20). Om hierin meer inzicht te krijgen zou meer onderzoek nodig zijn naar damgedrag en het effect van individuele verschillen in ademgedrag op de hoeveelheden uitgeblazen stoffen. Ook zou het interessant zijn om daarbij metingen te verrichten aan mogelijke variaties in dampgedrag die gebruikers vertonen in verschillende situaties of op verschillende dagen.

5.2 Uitgeademd volume

Alleen de eerste adem die wordt uitgeblazen na het nemen van een trekje is opgevangen en geanalyseerd. Slechts een deel van de lucht in de longen wordt hierbij uitgeademd, en hierin zijn verschillen tussen vrijwilligers, afhankelijk van ademgedrag en de fysiologie van de longen. De volumina van de adem die vrijwilligers op de filters uitbliezen is gemeten en varieerde van 33 mL tot 1414 mL per uitademing (gemiddeld over 5 uitademingen). Bij een klein volume betekent dit dat slechts een kleine fractie van de lucht uit de bovenste luchtwegen is uitgeblazen op het filter, terwijl bij de grote volumina juist een veel groter deel (waaronder lucht uit de diepere delen van de longen) is opgevangen en geanalyseerd. Deze waarden zijn niet typisch voor normaal ademgedrag in rust. Mogelijk ademen de vrijwilligers anders omdat ze aan het dampen zijn, of vanwege de experimentele omstandigheden ; ze moeten lucht uitblazen in een mondstuk waarbij ze merkbaar weerstand ervaren van het filter. Het is dus mogelijk dat er verschillen zijn in de hoeveelheden die dampers in de dagelijkse praktijk uitblazen en de door ons gemeten hoeveelheden als gevolg van verschillen in damp- en ademgedrag.

5.3 Toepasbaarheid op andere scenario's

In hoeverre de conclusies van scenario 1 (auto) en scenario 2 (kantoor) van toepassing zijn op andere scenario's, zoals een treincoupe of een café is afhankelijk van verschillende factoren. De concentraties van de uitgeademde stoffen in de lucht, en dus de gezondheidsrisico's voor omstanders, zijn sterk afhankelijk van onder andere het aantal dampers in een ruimte, de frequentie waarmee deze trekjes nemen, het volume dat wordt geïnhaleerd, hoe lang ze hun adem inhouden bij een trekje, het uitgeademde volume, het volume van de ruimte en de mate van ventilatie. Het is waarschijnlijk dat binnen dit grote bereik van verschillende condities ook situaties kunnen voorkomen waarin sprake is van gezondheidsrisico's, met name wanneer meerdere dampers zich in een kleine ruimte bevinden zoals een trein of een klein café.

5.4 Aantallen metingen

Voor de verschillende chemische analyses zijn niet dezelfde aantallen monsters gebruikt (bijvoorbeeld monsters van 3 vrijwilligers voor de analyse van metalen, 4 voor aldehydes, 9 voor TSNAs, en 17 voor nicotine, propyleenglycol en glycerol). Voor de risicobeoordeling is gebruik gemaakt van de hoogst gemeten waarde, maar dit is niet hoogste waarde die potentieel zou kunnen voorkomen. Naarmate meer vrijwilligers worden geïncludeerd neemt de kans toe dat een nog hogere waarde wordt gevonden. Dit is niet van belang als er nauwelijks spreiding in de gevonden waarden is, zoals bij de aldehydes het geval is. De gevonden hoeveelheden daarvan komen niet uit boven de hoeveelheden die ook al van nature voorkomen in adem (als geen e-

sigaret wordt gebruikt). Het speelt wel een rol bij de metingen die een grote spreiding laten zien, zoals bij propyleenglycol. Slechts bij 4 van de 17 vrijwilligers werden hiervan meetbare hoeveelheden aangetroffen, waarbij de hoeveelheid in het hoogste monster meer dan 4 keer hoger was dan bij het op-een-na hoogste monster.

5.5 Productvariatie: e-vloeistoffen en e-sigaretten

Er is een groot aantal verschillende e-vloeistoffen te koop in Nederland. Bovendien maakt een deel van de e-sigaret gebruikers zelf e-vloeistof, zoals ook blijkt uit het feit dat 33% van de vrijwilligers die deelnamen aan dit onderzoek aangaf een voorkeur te hebben voor een zelfgemaakte e-vloeistof. Voor dit onderzoek zijn e-vloeistoffen geselecteerd waarvan bij eerdere metingen was vastgesteld dat deze damp opleveren met relatief hoge concentraties van aldehydes, TSNAs, metalen en nicotine (een gedetailleerde beschrijving van deze selectie is te vinden in sectie 9.2.3).
De e-sigaretten die voor het onderzoek in dit rapport zijn geselecteerd zijn populaire modellen die goed verkrijgbaar zijn op de Nederlandse markt. Er bestaan e-sigaretten met meer vermogen die meer damp opleveren, maar die worden momenteel slechts gebruikt door een klein deel van de gebruikers. Als in de toekomst de populariteit van deze producten toeneemt, dan kan het nodig zijn om meer onderzoek te doen naar de hoeveelheden schadelijke stoffen die deze producten genereren, en de hoeveelheden die e-sigaret gebruikers daarmee uitademen. Daarbij moet dan ook rekening worden gehouden met de mogelijkheid dat het dampgedrag anders kan zijn omdat damp met hogere concentraties wordt geïnhaleerd, maar ook omdat dergelijke producten door een andere categorie gebruikers wordt aangeschaft.

5.5.1 Tabakspecifieke nitrosamines

Tabakspecifieke nitrosamines zijn soms aanwezig als verontreiniging in ingrediënten die door fabrikanten worden gebruikt bij de productie van e-vloeistoffen, met name in nicotine en in tabaksextracten die als smaakstof kunnen worden gebruikt. Bij gebruik van e-vloeistof die geen TSNAs bevat, zal de e-sigaret aerosol ook geen TSNAs bevatten en zal er dus ook geen sprake zijn van gezondheidsrisico's voor omstanders of gebruikers als gevolg van blootstelling aan deze stoffen. Voor het onderzoek dat is beschreven in dit rapport is gebruik gemaakt van e-vloeistoffen die een relatief hoge concentratie TSNAs bevatten om een '*worst-case*' risicobeoordeling te kunnen maken. De meeste e-vloeistoffen bevatten echter veel minder of geen TSNAs. Ook moet worden opgemerkt dat nieuwe wetgeving het gebruik van ingrediënten die onnodig verontreinigd zijn met stoffen die schadelijk zijn voor de menselijke gezondheid (zoals TSNAs) niet toestaat (24).

5.6 Onbekende risicos

Naast de stoffen die voor dit onderzoek zijn gemeten en waarvoor een risicobeoordeling is uitgevoerd bevatten E-sigaretten en de aerosol ervan nog andere stoffen, zoals smaakstoffen, conserveringsmiddelen en mogelijk nog onbekende verontreinigingen. Op dit moment zijn onvoldoende gegevens beschikbaar om daarvan een risicobeoordeling te kunnen uitvoeren. Meer onderzoek op dit terrein is wenselijk, gezien de voortdurende populariteit van e-sigaretten.

6 Conclusies

De prevalentie van e-sigaret gebruik in Nederland bedraagt ongeveer 1.4%. Van de dagelijkse e-sigaret gebruikers gebruikt ongeveer 78% een e-vloeistof met een nicotine concentratie van 6 mg/ml of meer. In overeenstemming met onderzoek van anderen bevestigen onze resultaten dat er grote verschillen zijn in het dampgedrag van individuele gebruikers (18, 20, 21, 25).
Een deel van de stoffen die voorkomen in de aerosol van e-sigaretten blijven achter in de luchtwegen van de gebruiker. Dit is van belang voor het evalueren van de blootstelling van omstanders, omdat e-sigaretten geen aerosol produceren op momenten dat er geen trekje wordt genomen. Omstanders worden daarom alleen blootgesteld aan stoffen die door e-sigaret gebruikers worden uitgeademd. Zowel uit ons onderzoek als uit door anderen gepubliceerd onderzoek blijkt dat er grote verschillen zijn tussen de samenstelling van e-sigaret aerosol en de uitgeblazen adem van e-sigaret gebruikers (14, 16). Het is daarom van belang dat bij de beoordeling van risico's van omstanders wordt uitgegaan van de samenstelling van uitgeblazen adem van e-sigaret gebruikers. In hoeverre verschillen in damp- en ademhalingsgedrag van gebruikers van invloed zijn op de hoeveelheden stoffen die gebruikers uitblazen is momenteel niet duidelijk.
De risicobeoordeling is uitgevoerd voor twee vooraf gedefinieerde scenario's (beschreven in 10.2.1). Kort samengevat gaat het in scenario 1 om een kind dat in een niet-geventileerde auto zit met twee e-sigaret gebruikers. Scenario 2 betreft een deel van een werkdag waarbij een volwassene in een kantoorruimte zit met een collega die een e-sigaret gebruikt. Als een nicotine-houdende e-sigaret gebruikt wordt dan bestaat in beide scenario's ten gevolge van de blootstelling aan nicotine een risico op hartkloppingen en een verhoogde systolische bloeddruk. De magnitude van de bloeddrukverhoging is vergelijkbaar met het effect van de inname van een dosis cafeïne uit twee of drie koppen koffie (5-14 mmHg).
Voor het 1^{e} scenario (auto) kan (milde) irritatie van de neus, keel oren optreden als gevolg van de blootstelling aan propyleenglycol. Indien de gebruikte e-liquid verontreinigd is met tabakspecifieke nitrosamines (TSNAs), dan kan een verhoogd risico op het ontstaan van tumoren in de luchtwegen niet worden uitgesloten voor het kind in scenario 1. Voor scenario 2 kan hierover geen uitspraak worden gedaan. Hierbij moet worden opgemerkt dat de meeste e-vloeistoffen geen of slechts lage concentraties TSNAs bevatten, en dat nieuwe wetgeving het voorkomen van TSNAs in e-vloeistoffen niet toestaat (24).
De gezondheidsrisico's voor omstanders zijn afhankelijk van de omstandigheden zoals het aantal e-sigaretgebruikers, het dampgedrag, de afmetingen van de ruimte, de mate van ventilatie van de ruimte en de duur van de blootstelling. Daarnaast is ook de samenstelling van de gebruikte e-liquid van grote invloed.
Behalve de stoffen die in het kader van dit onderzoek zijn gemeten en waarvoor de risicobeoordeling is uitgevoerd bevat e-sigaret aerosol nog andere stoffen zoals smaakstoffen, conserveringsmiddelen en mogelijk nog onbekende onzuiverheden. Hiervan zijn momenteel nog onvoldoende gegevens beschikbaar om een risicobeoordeling te kunnen

uitvoeren, en gezien de voortdurende populariteit van e-sigaretten kan het van belang zijn hiernaar in de toekomst verder onderzoek te verrichten.

Technical appendix

To provide additional detail with regard to the methods and results of the experimental research and the risk assessment based on it, the following chapters contain a technical appendix. In contrast to the main text, the appendix is written in English to make it accessible to an international audience and has been structured to allow it to be understood without need to read the preceding Dutch chapters.

Definitions and abbreviations

PG	Propylene glycol
TSNAs	tobacco-specific nitrosamines. This is a collective term for four compounds: N-nitrosonornicotine (NNN), N'x-nitrosoanatabine (NAT), N-nitrosoanabasine (NAB) en 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)
aerosol	The visible vapor generated by an e-cigarette that is inhaled by users.
exhaled breath	Used in this report to refer to the breath exhaled by someone that is using an e-cigarette

7 Technical appendix: Introduction

7.1 Introduction

Previous research, by us (1) and others (2), has shown that several components of the aerosol generated by e-cigarettes are present 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 for bystanders of e-cigarette use, performed by the Dutch National Institute for Public Health and the Environment (RIVM). The preceding chapters contain a summary of this information in Dutch. The risk assessment is limited to the toxicological risks due to exposure of bystanders to components of exhaled breath. Other potential effects such as for instance renormalization of smoking as a social norm (3, 4), injuries resulting from exploding e-cigarettes (5-7) or e-liquid ingestion and nicotine poisoning of infants (8-11) have not been considered.

7.2 Definitions: aerosol and exhaled breath

It is important to recognize that the composition of the aerosol that is produced by an e-cigarette and subsequently inhaled by the user (hereafter termed ‘aerosol’) is different from that of the breath that e-cigarette users exhale and that is distributed in the surrounding space (termed ‘*exhaled breath’* in this report) (14-16).This is not surprising, because, as an example, components of the aerosol are partially deposited or absorbed in the respiratory tract of e-cigarette users. Therefore, machine-generated e-cigarette aerosol should not be used as a substitute for exhaled breath of e-cigarette users to estimate the levels of harmful emissions that bystanders are exposed to.

7.3 Main-stream and sidestream smoke

The purpose of this report is to asses bystanders exposure to emissions from e-cigarettes, However, for the purpose of comparing our results to existing literature on this subject it is useful to consider an important difference between e-cigarettes and tobacco cigarettes.
Tobacco cigarettes continue to burn and produce smoke even when no puff is being taken. The smoke emitted during this phase is called sidestream smoke (SSS). The smoke that is inhaled by smokers is known as mainstream smoke (MSS). Sidestream smoke may contribute as much as 85% of the environmental tobacco smoke (ETS) that bystanders are exposed to (17).
In contrast, e-cigarettes do not produce the equivalent of sidestream smoke. Users only activate the heating element by pressing a button when taking a puff (or the device is activated automatically by an integrated airflow sensor). Accordingly, bystanders are only exposed to exhaled breath. If not accounted for, this difference between tobacco cigarettes and e-cigarettes will result in an overestimation of the exposure of bystanders of e-cigarette users (2).

7.4 Bystander exposure to exhaled breath

Others have also 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 which bystander exposure was estimated using machine-generated e-cigarette aerosol. As discussed above (section 7.2 and 7.3), this will result in an unrealistic overestimation.
However several papers have been published in which exhaled breath generated by vaping human volunteers was used. Fernandez *et al*. have recently published a systematic review on this topic (15). In the majority of these studies, test subjects vaped for some time in a test chamber. The air in the chamber was then sampled and analyzed. An overview of these studies is provided in table 7.1.

*Table 7.1: overview of publications pertaining to bystander exposure to components of exhaled breath based on vaping volunteers in a test chamber. The column 'measurements'' refers to the measurements that were performed of the air in the chamber. Abbreviations: CO – carbon monoxide ; <LOD – below level of detection; VOC – volatile organic compounds ; - PAHs – polyaromatic hydrocarbons. *) 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 (26)	60 m^3	3	20 puffs	particles
Schripp (27)	8 m^3	1	6 puffs in 6 minutes	VOCs particles aldehydes PG nicotine
Czogala* (28)	39 m^3	5	*ad lib.* twice for 5 minutes with 30 min. interval	nicotine particles CO (<LOD) VOCs (<LOD)
Ruprecht (29)	50 m^3	1	Two 7 minute sessions with 1 puff/min with 3 min. intervals	particles
Saffari (30)	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 (31)	45 m^3	3	2 hours	particles VOCs PAHs carbonyls metals

reference	test chamber volume	number of volun-teers	vaping pattern	measurements
O'Connel* (32)	12.8 m^3	3	3.2 puffs/min	humectants total VOC nicotine (<LOD) PG, aldehydes, isoprene, acetone, pentanesiloxane PAHs (<LOD) metals (<LOD) TSNAs (<LOD)

Study designs differ considerably among studies, e.g. 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 to apply them to different scenarios.

Ballbè *et al.* (33) took a different approach. They attempted to obtain a real-world assessment of nicotine exposure of bystanders by measuring nicotine levels in air at homes of e-cigarette users, and levels of cotinine in saliva and urine of members of the residence who did not smoke or use e-cigarettes. Even though nicotine levels in the air of the e-cigarette users homes were 5.7-fold lower compared to the air levels in the house of smokers, the cotinine levels in urine and saliva of non-smoking residents were comparable. The authors suggest that this discrepancy may be caused by the fact that the cotinine levels reflect actual exposure over a longer period prior to the measurement, while air sampling in the house was only done at a specific location. Furthermore, they indicate that they cannot completely exclude exposure of the subjects to cigarette smoke outside the context of the study. Some cotinine is also detected in the urine and saliva of the control group (which should not have been exposed to tobacco smoke or e-cigarette emissions). Furthermore, the authors did not study possible dermal exposure of the subjects.

Several authors performed chemical analysis of exhaled breath of e-cigarette users directly, using similar methods as were used for our report.
Long *et al. (14)*, found no detectable levels of carbonyls and phenolic compounds in exhaled breath 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 exhaled breath collected, the rest being water.
Interestingly, O'Connel *et al*. (16) found that >99% of nicotine is retained by test subjects when they inhale the e-cigarette aerosol, whereas 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. In agreement with these findings, a technique known among users as ‘stealth-vaping’ consists of holding the inhaled aerosol in the lungs for a few seconds to reduce the amount of visible vapor.

7.5 Exposure scenarios and setup of current study

E-cigarettes are used in a variety of situations (1). As mentioned (section 7.4), several studies have been published in which the levels of harmful components of in specific settings have been measured, for instance resembling a typical office. Fernandez *et al.* has recently published a systematic review on this topic (15). 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.
The data required for this calculation includes the amount of harmful components in exhaled breath and data regarding the vaping behavior of users. These were obtained experimentally.
To measure vaping behavior, we recruited experienced e-cigarette users and recorded their behavior (topography) while they were using their own e-cigarette and preferred e-liquid *ad libitum*.
For the subsequent chemical analysis of the exhaled breath, samples of the exhaled breath were collected close to their source, i.e. volunteers using an e-cigarette. The chemical analysis of exhaled breath 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 e-cigarette aerosol in our previous analysis, this class of components was not included in the present analysis.
The experimental data is then used to perform a risk assessment for the bystander of e-cigarette use. Hereto, two specific predefined scenarios of e-cigarette use were selected. The first scenario corresponds to an everyday car trip during which a child is exposed to chemicals in the breath exhaled by two e-cigarette users. The second scenario resembles exposure of an adult to breath exhaled by an e-cigarette user during part of a working day in an office room.

Chapter 8 and chapter 9 describe the experimental determination of vaping behavior and chemical analysis of exhaled breath, respectively. The risk assessment of the bystander of e-cigarette use is described in chapter 10. The general discussion and conclusions are presented in chapter 11 and chapter 12.

8 Technical appendix: 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 (18, 20, 25). It has been suggested that the differences in topography between vaping and smoking results from the fact that users regulate their uptake of nicotine from the aerosol (34). 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 (18) 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 (34).

8.2 Methods

8.2.1 Recruitment of test subjects

The study was reviewed and approved by the Medical Ethical Committee of Wageningen University (METC regnr. NL53471.081.15). E-cigarette users were identified in a screening 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, flavor and nicotine content of the e-liquid brought by the subject were recorded. The use of the CReSS pocket flowmeter (Borgwaldt, Hamburg, Germany) 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.

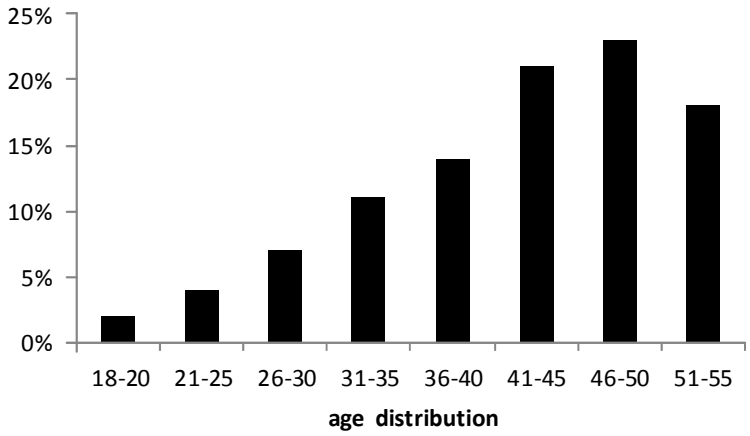
8.3 Results and discussion

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 participating in this study included 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%



Sixty-three percent of the daily e-cigarette users that used a nicotine-containing liquid was older than 40 years of age. The observed age distribution may be related to smoking cessation attempts (35).

8.3.2 E-cigarettes

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

Table 8.2: E-cigarettes and e-vloeistoffen 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-flavored liquid, while 10 subjects (56%) preferred a sweet flavor.

8.3.3 Topography

Eighteen test subjects participated in the experiment. As a result of malfunctioning of the CReSS pocket flowmeter, no useful data was recorded for the last three subjects. 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. Despite large variation observed in these parameters, the median values for the puff duration and volume calculated from all subjects are very similar to the values we previously inferred from available literature (4 sec puff duration, 55 mL volume) (1). The median puff interval was 43sec, which is also within the range of published values (20).

Table 8.3: Summary of vaping topography

	puff	puff interval (sec)			puff duration (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 topography was highly variable between subjects and between different puffs taken by one individual. However, the observed parameters are within the range of published values (18, 20, 25, 34).

9 Technical appendix: chemical analysis of exhaled breath of e-cigarette users

9.1 Introduction

In contrast to normal tobacco cigarettes, e-cigarettes do not produce an equivalent of sidestream smoke, ie. they only emit aerosol when the user takes a puff. As a result, bystanders are exposed exclusively to the breath exhaled by e-cigarette users. This chapter describes an analysis of the amounts of different chemicals in breath exhaled by volunteers during e-cigarette use.

9.2 Methods

9.2.1 Generation and sampling of exhaled aerosol

The same 18 test subjects recruited for the experiment in chapter 8 also participated in this experiment. Each participant was provided with an e-cigarette containing a mild tobacco-flavored liquid (section 9.2.2 and 9.2.3 describe details of the e-cigarettes and liquids used). The e-cigarette was connected to a CReSS pocket flowlogger (as described in chapter 8). A newly purchased clearomizer or cartomizer was used for every subject. The clearomizers were filled with approximately 1 mL of e-liquid at least one hour prior to the start of the experiment, or, in the case of the 1st generation device ('cig-a-like'), fitted with a new, unused cartomizer. The battery was fully charged for each subject. The clearomizers and cartridges were weighted immediately before and after the experiment to evaluate e-liquid consumption.

Subjects were explained the use of the breath 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 breath 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. To account for chemicals that occur in exhaled breath of subjects when they are not vaping, samples of control breath were first collected on filters/cartridges immediately prior to collecting the sample of exhaled breath during e-cigarette use. After taking the control breath samples, subjects were asked to take a specified number of puffs from the provided e-cigarette and after each puff the exhaled air of the first exhalation was collected 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 analyzed. The amounts trapped by the collection devices were normalized with respect to the volume of breath exhaled onto each filter. The concentration of the components found in the control breath samples were subtracted from those in the exhaled breath during e-cigarette use.

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

First generation e-cigarettes often contain notable levels of different metals, some of which also occur in the aerosol (1, 36). Therefore, the 1st generation e-cigarette selected (section 9.2.2) represents the sample with high metal content. Tobacco-flavored 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. The declared nicotine concentrations of these liquids were 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 4 mm glass fiber pads (commonly known as Cambridge Filter Pads (CFP)). We previously found that the filters trap these compounds effectively, and that the

compounds of interested be extracted for analysis with a high recovery. For 5 puffs, the first breath exhaled after drawing each puff was collected on a filter. Analytes were extracted from the filters with 15 mL of methanol containing 1,3-butanediol and heptadecane as internal standards for humectants and nicotine, respectively. The amount of the humectants was analyzed using GC-FID in accordance with WHO TobLabNet SOP6. The amount of nicotine collected was measured using LC-MSMS. Calibration curves were linear in the range of interest and made according to TobLabNet SOP6 and ISO10315 for the humectants and nicotine respectively.

9.2.5 Tobacco-specific nitrosamines (TSNA's)

Cambridge Filter Pads were used for collecting TSNA's. For 25 puffs, the first breath exhaled after drawing each puff was collected on a filter. After sample collection, a mixture containing all four stable-isotope labelled TSNAs was added to the filter 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 analyzed using LC-MSMS. The recovery of all four TSNAs from the filters using this method was >95%. A calibration curve was used to calculated absolute amounts, and was linear in the range of interest.

9.2.6 Aldehydes

Aldehydes were trapped using a filter holder containing a cartridge with carboxen-572 beads followed by a Cambridge Filter Pad, according to the method described by Uchiyama *et al* (37), with the following modifications. To reduce the air flow restriction presented by the carboxen cartridge, the plastic fritted disks at each end were replaced by fine gauze stainless steel. For 25 puffs, the first breath exhaled after drawing each puff was collected onto the 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 0.5 mL sample of the extract was derivatized with 0.2 mL of 2,4-di-nitrophenylhydrazine (DNPH), and diluted with 4.3 mL of ethanol. For all samples, a blank sample of the methanol/carbon disulfide solvent mixture was analyzed in parallel and subtracted from the samples. Absolute amounts were calculated using a calibration curve prepared using pre-derivatized aldehyde-DNPH analytical standards purchased from Sigma-Aldrich (Zwijndrecht, the Netherlands). Calibration curves were linear in the range of interest.

9.2.7 Metals

Cambridge Filter Pads were found to contain considerable and variable amounts of metals and therefore unsuitable 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 samples for the analysis of the metal content of exhaled breath.For 25 puffs, the first breath exhaled after drawing each puff was collected on a filter 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 30 mL and samples were analyzed using ICP-MS. Blank filters were analyzed in parallel to correct for metal content of the filters.

9.3 Results and discussion

9.3.1 Amounts of different components in exhaled breath

The amounts of propylene glycol, glycerol and nicotine were measured in the exhaled breath produced by 17 out of 18 subjects. The other components were measured in samples from a smaller 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)

Table 9.1: Summary of chemical analysis of exhaled breath. The values listed under 'amount' are the average amounts recovered in the first exhaled breath after inhaling a puff, the 'concentration' is the average 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. *) averages were calculated over the number of exhaled breaths collected on each filter (5 for nicotine, propylene glycol and glycerol, 25 for the other analytes)*

		amount				**concentration**			
		range				**range**			
	n	min	max	**median**		min	max	**median**	
nicotine	17	<LOQ	2140	108	ng	<LOQ	12391	323	ng / L
humectants									
propylene glycol	17	<LOQ	127	<LOQ	μg	<LOQ	839	64	μg / L
glycerol	17	<LOQ	<LOQ	<LOQ	μg	<LOQ	<LOQ	<LOQ	μg / L
nitrosamines									
NNN	9	<LOQ	111	29	pg	<LOQ	961	84	pg / L
NAT	9	<LOQ	40	14	pg	<LOQ	172	47	pg / L
NAB	9	<LOQ	8	2	pg	<LOQ	16	9	pg / L
NNK	9	<LOQ	71	15	pg	<LOQ	403	39	pg / L
aldehydes									
formaldehyde	4	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
acetaldehyde	4	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
acroleine	4	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
metals									
arsenic	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
molybdenum	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
tin	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
cadmium	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
lead	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
zinc	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
copper	3	<LOQ	2.92	<LOQ	ng	<LOQ	28	<LOQ	ng / L
nickel	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
cobalt	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
manganese	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
chromium	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
vanadium	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
uranium	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L

		amount				concentration			
		range				range			
	n	min	max	median		min	max	median	
nicotine	18	<LOQ	2140	108	ng	<LOQ	12391	323	ng / L
humectants									
propylene glycol	18	<LOQ	127	<LOQ	µg	<LOQ	839	64	µg / L
glycerol	18	<LOQ	<LOQ	<LOQ	µg	<LOQ	<LOQ	<LOQ	µg / L
nitrosamines									
NNN	9	<LOQ	111	29	pg	<LOQ	961	84	pg / L
NAT	9	<LOQ	40	14	pg	<LOQ	172	47	pg / L
NAB	9	<LOQ	8	2	pg	<LOQ	16	9	pg / L
NNK	9	<LOQ	71	15	pg	<LOQ	403	39	pg / L
aldehydes									
formaldehyde	4	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
acetaldehyde	4	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
acroleine	4	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
metals									
arsenic	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
molybdenum	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
tin	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
cadmium	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
lead	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
zinc	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
copper	3	<LOQ	2.92	<LOQ	ng	<LOQ	28	<LOQ	ng / L
nickel	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
cobalt	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
manganese	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
chromium	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
vanadium	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L
uranium	3	<LOQ	<LOQ	<LOQ	ng	<LOQ	<LOQ	<LOQ	ng / L

Nicotine was detected in all samples except one. The control breath samples also contained small amounts of nicotine, possibly from nicotine exposure prior to the experiment. These were subtracted from the amounts observed in the samples of exhaled breath during e-cigarette use.

While propylene glycol was observed in the exhaled breath from 4 out of 17 subjects, glycerol remained below the limit of quantification. The e-liquids used do contain glycerol, but its concentration in the liquid is 2 to 4-fold lower than that of propylene glycol. 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 in exhaled breath during e-cigarette use were below the limit of quantification in all 4 samples when corrected for

background levels. E-cigarettes are known to produce small amounts of different aldehydes during normal use (38), but these chemicals 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 exhaled breath samples collected during e-cigarette use did not exceed the levels observed in control breath samples.
The volume of exhaled breath produced by the test subjects onto the sample collection assembly was measured with a flow meter, to allow conversion between absolute amounts and concentration. There is considerable variation between subjects in this respect (ranging from average volumes of 33 mL to 1414 mL per exhalation, see also http://www.rivm.nl/bibliotheek/rapporten/2016-0036bijlage.pdf) and these values are not representative for normal exhalation or breathing volumes, presumably because subjects were required to exhale via a mouthpiece into the trapping assembly.

9.3.2 Comparing the composition of e-cigarette aerosol and exhaled breath

From the amount of e-liquid consumed during the experiments, an estimate was made of the amount of PG and nicotine recovered in the first exhaled breath as a percentage of the total amount vaporized. Our previous experiments indicated that the concentration of propylene glycol and nicotine in the vapor is proportional to the concentration in e-liquid and the amount of e-liquid that is vaporized (1). If we assume that the amount of e-liquid consumed in each puff was roughly constant throughout the experiment, the amount inhaled of these components can be calculated. For TSNAs, this is not possible, because these compounds do not transfer into the aerosol quantitatively. It is therefore not possible to calculate the inhaled amount from the known concentration in the e-liquid for the TSNA's. As shown in table 9.2, the absolute amount of nicotine and PG recovered in the first breath exhaled after taking a puff was only a small fraction of the vaporized amount. For 15 out of 17 subjects, this amounted to less than 1%.
It should be noted that the collected amounts are not equal to the total amounts that are exhaled. The inhaled air will mix with the air present in the lungs (*i.e.*, the functional residual capacity) and upon exhalation a fraction of the inhaled chemical will remain in the lungs and can be exhaled during subsequent breathing cycles. If no deposition or pulmonary absorbtion or takes place, the amount exhaled in the first exhalation is typically approximately 40% of the total amount (see section 10.4). 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 the humectants and nicotine are partially retained in the respiratory tract of the e-cigarette user and only a (small) fraction is exhaled.

Table 9.2: Recovery of propylene and nicotine in the first exhalation after taking a puff, expressed as a percentage of the amount that was vaporized and inhaled.

	% recovered in exhaled breath	
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	<LOQ	0.002
max	2.35	4.741
median	<LOQ	0.158

Similarly, the amounts of glycerol and aldehydes in exhaled breath 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 exhaled breath that has been sampled immediately upon exhalation in a similar fashion as in our investigation (14, 16, 39). In line with our findings, it was observed that only a small fraction of nicotine, propylene glycol and glycerol present in e-cigarette aerosol was recovered in exhaled breath.

Interestingly, O'Connel *et al.* (16) 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 air of a test chamber in which e-cigarette users exhaled, but differences in the experimental setup makes it difficult to compare those with our results. Fernandez *et al.* (15) has recently conducted a systematic review of the literature on this topic.

9.4 Conclusions

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

10 Technical appendix: Assessment of health risks of bystanders of e-cigarette users

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 breath (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 7.4.

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 behavior of the vaper and the pulmonary retention of the chemical. On the other hand, the concentration will decrease e.g. as a result of inhalation and pulmonary deposition or 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 (pulmonary) retention (*i.e.*, deposition and absorption) of the chemical. Since only the first exhaled breath following a puff was captured and analyzed, 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). The 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 (22).

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 tumors is considered to be very low following the EFSA approach (23). 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 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 vapor 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 (1) .

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 (1) .

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.

10.3 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 vapor. This calculation requires additional information (*e.g.*, on pulmonary retention 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 retained in the respiratory tract. The higher this fraction, the lower the amount of a chemical to be exhaled and thus the lower the bystander exposure. Retention includes deposition in the respiratory tract and absorption into the systemic circulation. Deposition may take place over the entire respiratory tract, depending on among others particle size and depth of inhalation. Gasses and vapors generally will enter the alveoli where gas exchange takes place. As a first worst-case approximation, it is assumed that retention only occurs in the deeper lungs and not in the so-called dead-space volume. This may underestimate the retention and thus overestimate the exposure of the bystander. For the risk assessment of systemic effects, it is further assumed that all deposited chemical is absorbed.
These assumptions mean that 100% retention 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 retention is therefore maximally 0.7.

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 can 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 pulmonary retention. In the present scenarios, a low retention would result in high air concentrations. Preferably, a chemical-specific value is to be used but often not available. Therefore, a pulmonary retention 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 retention 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 retention 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 retention than for local effects. 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 a retention of 50%; 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 retention of 50% is therefore used as a first, worst-case estimate for evaluation of systemic effects of the bystander. It should be noted that a *pulmonary* retention of 50% corresponds to an *alveolar* retention 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 retention.

Pulmonary retention 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 analyzed 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*. (40) with slight adaptations for vaping of e-cigarettes. Estimations are made separately for local effects on the respiratory tract and systemic effects as different estimates for pulmonary retention 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 is assumed to 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 retention 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 analyzed 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 retention fraction of 0.71 (*i.e.*, equivalent to a pulmonary retention 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 analyzed 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 retention fraction*	0.5
Alveolar retention fraction*	0.71

* values used for evaluation of systemic effects; for evaluation of local effects on respiratory tract a retention 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 retention fractions: no retention, low retention (0.3), high retention (0.71) and full (100%) retention. As indicated in figure 10-1, in case of high retention (red curve) and full retention (green curve), the alveolar concentration has decreased to zero before drawing a new puff. Thus, starting from a pulmonary retention fraction of 0.5 (equivalent to an alveolar retention 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 retained, 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 retained, *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.

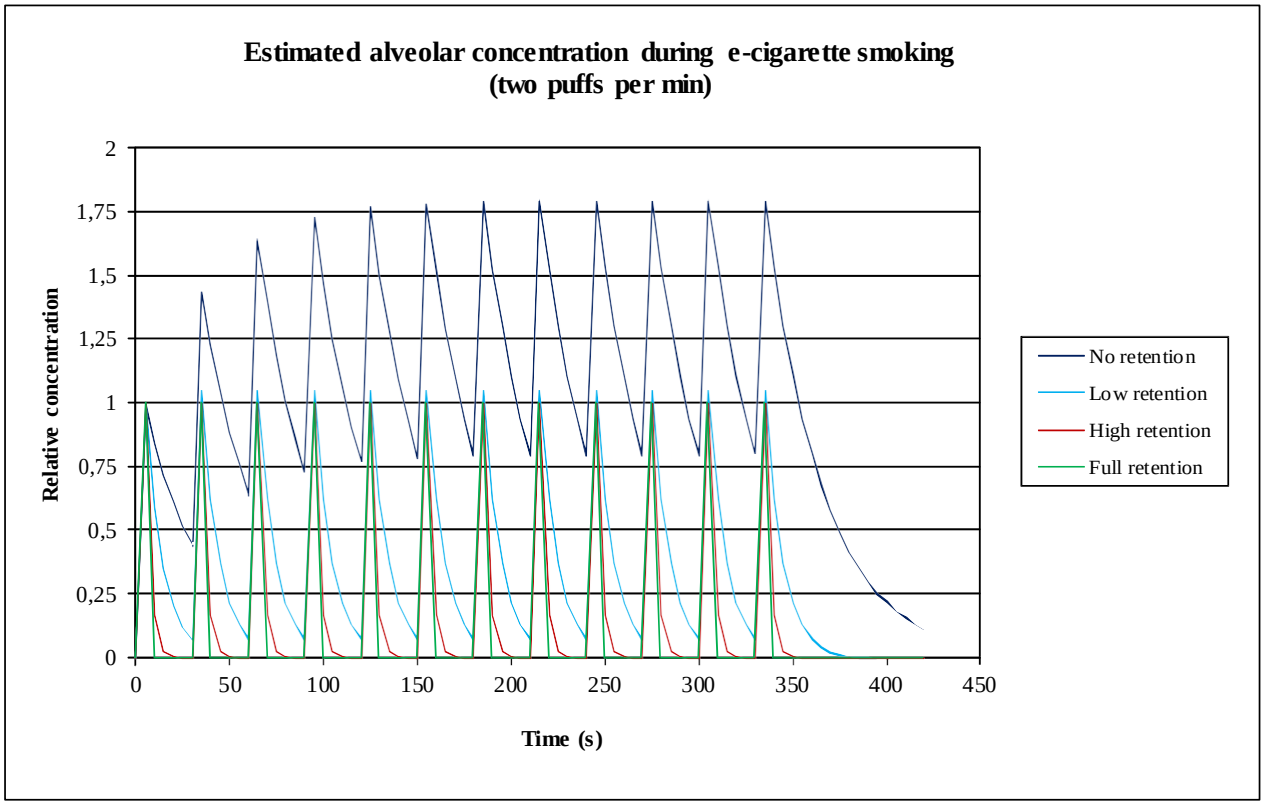


Figure 10.1 Estimated relative alveolar concentration during an e-cigarette vaping session of 6 minute duration with a puff frequency of 2 min^{-1}. Dark blue line ('no retention') corresponds to an alveolar retention fraction of zero; light blue corresponds to an alveolar retention fraction of 0.3;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

10.3.1 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:

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

conc = concentration of a chemical in room upon vaping (mg/m^3)
$F_{pulm,ret}$ = pulmonary retention 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:

$D_{syst} = conc \times F_{pulm,ret} \times t \times RV$
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) *
$F_{pulm,ret}$ = pulmonary retention fraction
RV = respiratory volume (default: 0.2 l/min/kg bw for a 70 kg person according to ECHA (2012) (41), 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 (42))
** 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 (43) (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.
Default values on retention were used. As a first worst-case estimate for evaluation of local pulmonary effects of the bystander, a pulmonary retention fraction of zero is used. A higher pulmonary retention will reduce the exposure concentration, *e.g.*, a retention 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.

10.4 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.

10.4.1 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.

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 hemorrhage with a LOAEL of 160 mg/m^3 ((44) as described in (45)).
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 ((46) as described in (47)). 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 ((44) as described in (45)); 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 (48).

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.

[1] The inhalation exposure is converted to an equivalent systemic dose, based on a respiratory volume of 1.29 m^3/kg bw for the rat for a 6-h exposure (ECHA, 2012)

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 retention 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 and eyes 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.

Additionally the use of a default retention 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.

10.4.2 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.
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) (49, 50).
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 ((51) as decribed in (49)). 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 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 ((52) as described in (49)). 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

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

inhalations), resulting 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 ((52) as described in (49)).
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 retention 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 ((52) as described in (49)) (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 of 2.1 and of 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 retention 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

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/m3 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 retention fraction of 0.5 and a bw of 70 kg.

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 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 ((53) as described in (49)).
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.

10.4.3 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.

10.4.4 Risk assessment tobacco-specific nitrosamines (TSNAs)

Exposure

Four tobacco-specific nitrosamines were analyzed: 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.
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 tumors in experimental animals; in general this considers lung tumors independent of the exposure route (54). 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 (55). 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 (54).
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 tumors in the nasal cavity. These effects are considered relevant for the potency to induce respiratory tract tumors 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 tumor incidences for the nasal tumors were 0/36, 13/36, 31/36, 19/36, respectively ((56) as described in (57)). 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 (1) . The analysis resulted in a BMDL10 of 3 µg/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 tumor development upon exposure to tobacco-specific nitrosamines, independent of the exposure route (54). Whether these tumors 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 tumors were observed is used for risk assessment. It is assumed that these tumors 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 (µg/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 tumors is very low ((23); 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 total amount of TSNAs in 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 (54). 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 retention 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 tumor incidences cannot be estimated as data concerning this issue are lacking. For the time being, it is assumed that increased incidences of tumors 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 retention 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 tumors is very low, but this cannot be stated with sufficient certainty.

10.5 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.
Scenario 1 - car: For risk assessment, a worst-case exposure concentration (assuming no pulmonary retention) 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 retention) 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 (58). 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, molybdenum, 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, molybdenum, 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.

10.6 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 and eyes upon exposure to propylene glycol may occur for the bystander of e-cigarette vaping. However, it is expected that effects, if they occur, will 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 tumors 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. It is noted that Hanson *et al.* (1994) describe concentration-response relationships for the effects of nicotine on the systolic blood pressure and heart rate. The magnitude of the effects on blood pressure at the PoD are quantitatively similar as is described for a single dose of caffeine equivalent to two or three cups of coffee (5-14 mm Hg), with higher increases observed in hypertensive persons (59). The increase in heart rate at the PoD was up to 10 beats/min. 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 low concern’, which means that the risk for tumors is very low, 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, breath holding following a puff, depth of inhalation, 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 vapor. 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 vapor 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 intra-individual variation (see chapter 8), affected this measured amount in the exhaled air and that was used for risk assessment.

11 Limitations

In order to identify possible health risks of e-cigarettes to bystanders, two scenarios were defined in which relatively high levels of exposure to bystanders occur but that are not unrealistic. The initial finding that a significant fraction of the inhaled nicotine and propylene glycol is retained by the users (and presumably other components of the e-cigarette aerosol as well), and the fact that the exhaled breath will be strongly diluted into the surrounding air prompted us to focus on these realistic worst-case scenarios. Should the risk assessment indicate that no significant health risks occur even in these scenarios, it would then be possible to extend this conclusion to other (less worst-case) scenarios.

The scenarios are outlined in section 10.2.1. Briefly, scenario 1 resembled a daily car trip during which a child is exposed to chemicals exhaled by two e-cigarette users. 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 these scenarios, health risks were identified.
For scenario 1, irritation of the upper respiratory tract may occur as a result of exposure to propylene glycol. For both scenarios, exposure to nicotine may result in increased heart rate, increased systolic blood pressure. If liquids with a high level of tobacco-specific nitrosamines (TSNAs) are used, an increased risk of tumors in the respiratory tract cannot be excluded (it should be noted that new European regulation (24) does not allow the presence of TSNAs in e-liquids).

As discussed below, the risk assessment includes uncertainties due to the assumptions and choices that were made. The risk assessment was based on the highest amount exhaled for each chemical, with nicotine, propylene glycol and TSNAs being the most important ones in the present evaluation. For propylene glycol, which was below the LOQ in exhaled breath for most subjects, effects on eyes and upper respiratory tract may occur but are expected to be minor, if any. As to nicotine, estimation of bystander exposure based on the by far highest exhaled amount of nicotine would result in increases in heart rate and in blood pressure that are comparable with the effects of caffeine present in two to three cups of coffee.

For the present risk assessment bystander exposure was estimated from the amounts in the exhaled breath immediately following a puff and models of respiration and distribution of chemicals into the environment. The design of the experimental studies, uncertainties in the data and assumptions made about respiration physiology may impact the risk assessment. The present chapter elaborates on the main uncertainties relating to the experimental conditions and the risk assessment.

11.1 Variation in vaping topography and respiration behaviour

Since the only source of bystander exposure consists of the chemicals exhaled by the vaper, parameters relating to the inhalation (the puff) and the subsequent exhalation may affect our estimate of bystander exposure. These include puff volume, puff interval, total volume inhaled with a puff (shallow or deep inhaling), breath holding following a puff, total volume exhaled, etc. All these parameters may affect the amount exhaled in different ways. It is beyond the scope of this study to systematically evaluate how the combination of these parameters affect the exhaled amounts. Furthermore, insufficient data is available on the typical and extreme values representative of natural vaping behavior. However, the volunteers in our study were allowed to vape and breathe during the experiment as they preferred, and the data used for the risk assessment therefore comprises a sample of the range of vaping and exhalation behavior, although it is likely that exhalation behavior during the experiment conditions is different from normal breathing behavior (see also 11.1.1). Large inter- and intra-individual variation in vaping behavior was observed (see chapter 8), in agreement with similar studies performed by others (20). It may be of interest to study specifically the effect of breathing and vaping behavior on the amounts exhaled by e-cigarette users in the future, and to acquire additional data on vaping behavior. Furthermore, it would be interesting to examine variations in vaping topography in a single subject on different days or in different situations.

11.1.1 Inhalation

The depth of inhalation may affect the exhaled amount. For instance for nicotine it was shown by Armitage *et al*. that the extent of retention is dependent on the extent of inhalation (60). Volunteers were asked to take a puff from a cigarette and after each puff either hold the smoke within their mouth, inhale with a fixed volume of 75 ml or inhale deeper with a volume of 500 ml. Following inhalation breath was held for two seconds. It was shown that the percentage nicotine retained was highest under conditions of deep inhalation (60) (up to 100%) whereas approximately 50% was exhaled when smoke was held within the mouth. This suggests that with shallow inhalation of tobacco smoke, a larger percentage of nicotine will be exhaled. Similar findings were reported for e-cigarettes (16). In the present risk assessment for systemic effects a retention of 50% was used as a worst-case default value. Considering the much higher alveolar retention of for instance nicotine this would have led to an overestimation of the bystander exposure. However, if a vaper only holds the inhaled aerosol in his mouth, approximately 50% of the inhaled nicotine might be exhaled. A bystander then will inhale the nicotine deep into the lungs with a retention of approximately 100%. Thus a default retention of 50% for nicotine will overestimate bystander exposure if the vaper inhale the aerosol deep into the lungs but may slightly underestimate bystander exposure if the vaper holds the aerosol within the mouth. The underestimation is due to the fact that the default retention of 50% is an underestimation of the retention of 100% for the bystander. However, the fraction of vapers that do not inhale into the deeper lungs is unknown, and it can therefore not be verified whether or how often this situation will occur in practice. A similar reasoning might also hold

for other components of an e-cigarette aerosol but data are not available.

11.1.2 Puff interval, volume and duration

If the interval between puffs is very short, it is possible that a small amount of material from the previous puff is retained by the user and exhaled and collected during the next exhalation on the filter. However, because the interval between puffs was more than 1 min for all puffs during the experiment, we expect such carry-over to be negligible.
The puff volume and puff duration may also be of relevance. During the assessment of the topography prior to the study a large intra- and interindividual variation was observed for puff volumes and puff durations. Pilot experiments revealed that both puff volume and duration have an effect on the amount of liquid that is vaporized ((1) and not shown), with higher volumes and duration resulting in the evaporation of more e-liquid. The median puff volume between subjects ranged from 20.1 to 121.8 ml whereas the minimal and maximal volumes for individual subjects differed up to a factor of 10 (and even up to 20 in one subject). The puff duration ranged from 5.0 sec to 17.4 seconds and a difference between minimal and maximal duration of a factor of 21 for one subject. The amount of chemicals inhaled, and probably also the amount subsequently exhaled, is therefore related to the puff volume and duration. It is not known whether the puff volume and duration for the individual volunteers during the experiment was representative for their everyday vaping behavior.

11.1.3 Exhalation

Only the first exhalation immediately following inhalation of aerosol from the e-cigarette was collected onto the sample collection devices and analyzed. The fraction of the total amount of air in the lungs that is exhaled is variable between subjects and dependent on breathing behavior and lung physiology. The participants were free to breathe and vape as they preferred in terms of breathing volume and puff volume, puff interval and puff duration. The volumes exhaled onto the filters were recorded and found to be highly variable, with values ranging from 33 mL to 1414 mL for one exhalation (averaged over 5 exhalations). These extremes suggest that the exhalation behavior of some subjects under the experimental conditions is different from normal breathing behavior, and possibly also from their normal breathing behavior while vaping. A normal exhalation volume in rest would be approximately 500 mL. For low volumes this means that only a small fraction of the air present in the upper airways is exhaled onto the filter, whereas in the case of high volumes, a much larger fraction, including air from the deeper lungs, is collected and analyzed. The extremes of the observed range in exhaled volumes are not typical for normal breathing behavior in rest. It is possible that breathing behavior is different when using an e-cigarette or that subjects altered their breathing behavior because of the experimental conditions; they had to exhale into a mouthpiece onto a filter that noticeably restricts air flow. For instance, an exhaled volume of 33 ml is merely a puff, which may have resulted in an underestimation of bystander exposure. Thus, considering the possibility that the vaping and exhalation behavior of the volunteers during the experiment may be different from their normal behavior, the exhaled amounts of chemicals, and therefore the estimated bystander exposure,

may be different in daily practice. For the risk assessment, default values were used for the parameters related to breathing behavior and lung physiology (table 10.3)

In this context, it is of interest to examine the relationship between the amounts of chemicals exhaled and the average volume of the exhaled breath for the individual subjects. As shown in figure 11.1, no clear correlation between these values was observed for nicotine (left panel), propylene glycol (middle panel) and for total TSNAs (right panel). The largest amounts of propylene glycol, nicotine and second-largest amount of total TSNAs were observed for one subject that exhaled relatively large volumes onto the filters. In contrast, a subject with an approximately similar exhalation volume exhaled a 50-fold lower amount of nicotine and the exhaled amount of propylene glycol was below the LOQ (figure 11.1, left and middle panel), despite the fact that this subject used an e-liquid with a higher nicotine content (18 mg/ml). Furthermore, the largest amount of total TSNAs was exhaled by a subject with an approximately threefold lower exhalation volume (figure 11.1, right panel). This highlights the impact of the vaping topography and the respiration physiology on the exhaled amounts.

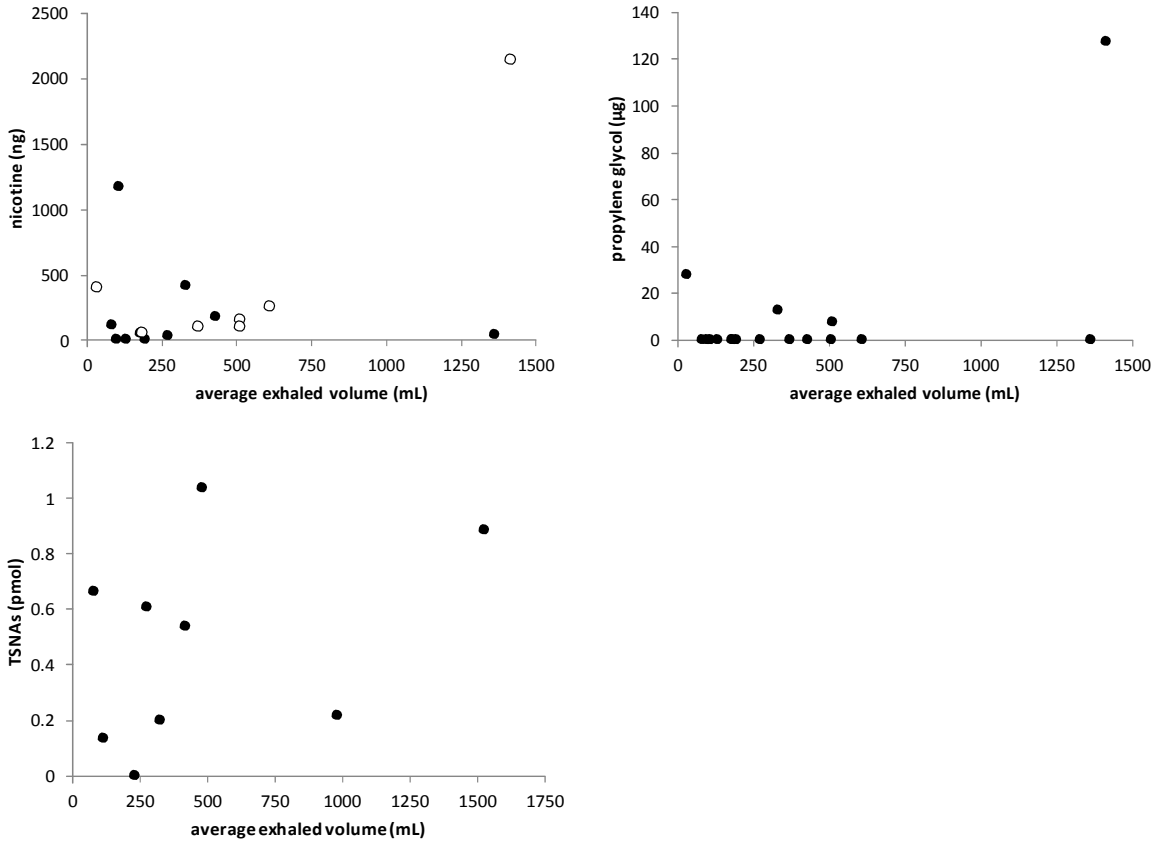


Figure 11.1 relation between collected amounts of nicotine, propylene glycol and total TSNAs on the trapping assembly to the exhaled volume. For nicotine (left panel), open and filled symbols indicate the nicotine concentration of the e-liquid used by the volunteers (11 and 18 mg/ml, respectively)

11.2 Extension to other scenarios

Whether the conclusions drawn for scenario 1 (car) and scenario 2 (office) would also apply to other exposure scenarios in public spaces such as a compartment of a train or a pub is dependent on many factors. The level of the indoor air concentrations, and subsequently the resultant health risk, are highly dependent on the number of persons vaping, their puff-frequency, the total vaping time, puff volume, puff interval, total volume inhaled with a puff (shallow or deep inhaling), breath holding following a puff, volume of exhaled air, the volume of a room, and the extent of ventilation. In this wide range of different conditions, it is likely that health risks may occur for bystanders in other scenarios as well, especially in situations in which multiple vapers are present in a relatively small space such as a train or small pub.

11.3 Sample size

The number of volunteers was not identical for the different chemical analyses (e.g., 3 volunteers for the analysis of metals, 4 volunteers for the analyses of aldehydes, 9 volunteers for TSNA analysis and 17 volunteers for nicotine and humectants analyses). A larger dataset will contain more extreme values. For the present study, the highest measured amounts were used but this may not represent the actual worst-case situation. Possibly, higher amounts might have been found if more vapers had been included. However, this is not a concern when the variability in the data is small, such as for aldehydes of which amounts in exhaled breath did not exceed naturally occurring amounts in control breath. However, it should be considered for data showing a high variability, for instance in amounts of exhaled PG which only occurred in detectable amounts in the exhaled breath 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 based on the highest amount exhaled for each chemical, with nicotine, propylene glycol and TSNAs being the most important ones in the present evaluation. From figure 11.1 it can be concluded that, at least for nicotine and propylene glycol, the highest amount was by far the highest amount measured indicating that the present risk assessment has a relatively high worst-case character.

11.4 Product variability: e-liquids and e-cigarettes

A very large number of different e-liquids are commercially available in the Netherlands. In addition, a significant number of users prepare their own e-liquids, as evidenced by the fact that 33% of the volunteers in our study preferred home-made e-liquid. As described in section 9.2.3, e-liquids were selected that we previously found to yield relatively high vapor concentrations of aldehydes, TSNAs, metals and nicotine. Of the three e-liquids used for the current study, one contained 11 mg/ml of nicotine, while the other two liquids contained 18 mg/ml nicotine (the maximum currently allowed by Dutch legislation is 20 mg/ml). Therefore, we expect the amounts inhaled and subsequently exhaled by the vapers to be relatively large.
The e-cigarettes selected for the experiments are models that are currently popular and widely available on the Dutch market. More powerful models of e-cigarettes exist that can generate more vapor, but these are currently only used by a small fraction of users. Should the

popularity of these devices increase significantly in the future, it may be necessary to evaluate the amounts of chemicals exhaled by users with these devices, taking into account that differences in vaping behavior may also occur, not only because user behavior may be different when inhaling a vapor with a much higher concentration of aerosol, but also because such devices may be bought by different types of users.

11.4.1 Tobacco-specific nitrosamines

Tobacco-specific nitrosamines in the vapor originate from the e-liquid. If e-liquid does not contain TSNA's, users and bystanders will not be exposed to these compounds. TSNAs may occur as impurities in ingredients used by manufacturers of e-liquids, most notably in nicotine, and tobacco-extracts used for flavoring. Most e-liquids currently available on the Dutch market do not contain significant amounts of TSNAs (1). For the current study, an e-liquid was selected that contained relatively high amounts of TSNAs to ensure a worst-case assessment. It should be noted that the new European Tobacco Product Directive does not permit the presence of compounds that are harmful to human health in e-liquids if this can be avoided (24).

11.5 Unknown risks

In addition to the chemicals present in the aerosol considered in this study, e-cigarettes typically contain flavorants, preservatives and may contain contaminants that have not been identified. For these chemicals, 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 (18, 20, 25, 34).
Part of the chemicals that occur in e-cigarette aerosol are retained in the respiratory tract of the user. This is of importance for evaluating the exposure of bystanders to chemicals that occur in e-cigarette aerosol, because in contrast to normal tobacco cigarettes, e-cigarettes do not produce emissions when the user does not take a puff. Bystanders are therefore only exposed to chemicals present in the exhaled breath of e-cigarette users. In light of the marked differences in the composition of e-cigarette aerosol and exhaled breath of e-cigarette users, observed by us and others (14) it is clear that any evaluation of the exposure of bystanders should be based on exhaled breath. It is currently still unclear to what degree individual differences in vaping and breathing behavior affect the extent to which different chemicals of the aerosol will be retained in the respiratory tract of the user, and how much is subsequently exhaled.
The risk assessment was performed for two predefined, realistic worst-case scenarios (outlined in section 10.2.1). Briefly, scenario 1 resembled a daily car trip during which a child is exposed to chemicals exhaled by two e-cigarette users. For this scenario, it cannot be excluded that local effects on the respiratory tract and eyes upon exposure to propylene glycol will occur for the bystander. However, it is expected that effects, if they occur, are mild. Furthermore, it should be noted that propylene glycol could not be detected in the exhaled air of 13 of the 17 volunteers. 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. The increase in heart rate is expected to be smaller than 10 beats/min and the increase in systolic blood pressure might be similar to that induced by caffeine when drinking two to three cups of coffee in these scenarios. In addition, vaping of e-cigarettes can result in increased indoor air concentrations of tobacco-specific nitrosamines, but this is highly dependent on the composition of the e-liquid used. For e-liquids containing significant amounts of TSNAs, increased incidences of tumors in the respiratory tract upon exposure to tobacco-specific nitrosamines cannot be excluded for the child in scenario 1. It should be noted that the new European Tobacco Product Directive (24) does not permit the presence of compounds that pose a risk to human health, such as TSNAs, in e-liquids if this can technically be avoided.
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. The extent of these increases are expected to be similar or lower than for scenario 1. With respect to the tobacco-specific nitrosamines, no firm conclusion was possible for scenario 2. The MOE value for the nitrosamines in this scenario was such that

refinement of the MOE calculation may lead to the conclusion ‘of low concern’, which means that the risk for tumors is very low, but this cannot be stated with sufficient certainty. Furthermore, most e-liquids contain only negligible quantities of TSNAs.
The risks for the bystander are strongly dependent on the exposure conditions, such as the vaping topography of the e-cigarette users, the numbers of vapers, the dimensions of the room, the extent of ventilation, and the duration of exposure of the bystander, etc,. Furthermore, the composition of the e-liquids used is an important factor in the exposure of bystanders.
In addition to the chemicals present in the aerosol considered in this study, e-cigarettes typically contain flavorants, preservatives and may contain contaminants that have not been identified. For these chemicals, 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 Appendix A: 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 behavior 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 retention (in case of local effects on the respiratory tract) or using a default retention 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 tumor 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	Reference
rat (f)	inhalation	20 h/d, 5d/wk, during 2 year	0.5 mg/m^3	Reduced bodyweight (5%); not clear whether this is statistically significant	Waldum et al., 1996
rat (m)	oral (via drinking water)	34 wk	1.14 and 4.56 mg/kg bw/d	Minimal biochemical changes in cardiac muscle a high dose level	Wenzel and Richards, 1970; via Health council of the Netherlands (2005)
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 behavior (such as increased locomotor activity)	Welzl et al., 1988; via Health council of the Netherlands (2005)
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 behavior (such as increased locomotor activity), increased brain concentrations of monoamines. Effects were reversible within 12-24h after final dosing on day 50	Gäddnäs et al., 2000; via Health council of the Netherland

Species + sex	Exposure route	Exposure duration	Dose or concentration	Effects observed	Reference
					s (2005)
rat (m)	Subcutaneous via mini-osmotic pump	3 wk	1 mg/kg bw/d	Immunological effects (inhibition of Concavalin A-induced proliferation of periferal blood and spleen cells)	Singh et al., 2000; via Health council of the Netherlands (2005)
muis (f)	Subcutaneous	2e or 3e tremester of gestation	2.7 mg/kg bw/d	Reduced gestational period Maternal toxicity not described	Nasrat et al., 1986; via ACGIH 1994
rat (f)	Subcutaneous	GD 4-20	6 mg/kg bw/d	Reduced bodyweight dams and pups Reduced brain development pups Maternal toxicity not described	Slotkin et al., 1986; via ACGIH 1994
rat (f)	dermal (via transdermal patches)	GD 2-19 or GD 2-7	0 – 1.75 – 3.5 mg/d	Abortus at both experimental dose levels Fetuses not evaluated Maternal toxicity not described	Witschi et al 1994; via Health council of the Netherlands (2005)
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 pups Reduced body weight male pups Reduced locomotor activity male pups No effects on female pups Maternal toxicity not described	Peters en Tang, 1982; via Health council of the Netherland

Species + sex	Exposure route	Exposure duration	Dose or concentration	Effects observed	Reference
					s (2005)
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 water consumption during lactation period in dams Reduced number of pups per litter Reduced pup body weight during postnatal day 20, 30 and 40	Carr et al., 1985; via Health council of the Netherlands (2005)
rat (f)	oral (gavage)	GD1-21	0 and 3 mg/kg bw/d	Reduced body weight and food consumption with dams Reduced birth weight pups and smaller litters (not significant)	Leichter 1991; via Health council of the Netherlands (2005)
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 described	Rowell and Clark 1982; via Health council of the Netherlands (2005)
muis (f)	oral (gavage)	GD8-12	0 and 35 mg/kg bw/d	Maternal toxicity: overt signs of toxicity, reduced bw, lethality ratio of 10/26 Number of pups per litter, live pups on postnatal day 1-3, pup weight on postnatal day 1-3 were slightly higher, though no statistically significant.	Seidenberg et al., 1986; via Health council of the Netherlands (2005)

Species + sex	Exposure route	Exposure duration	Dose or concentration	Effects observed	Reference
rat (f)	subcutaneous	GD1-20	0 and 0.5 mg/kg bw/d	Pups: reduced pup bw, delayed eye opening postnatally, decreased sensory motor reflexes stimulation Early adulthood: stimulation motoric activity	Ajarem et al., 1998; via Health council of the Netherlands (2005)
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 ossification of sternae and skull when compared with pair-fed controls Maternal toxicity not described	Nash en Persaud 1989; via Health council of the Netherlands (2005)
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 (such as heart, brain, ear and eyes, hind limb) at high nicotine dose Maternal toxicity not described	Daeninck et al., 1991; via Health council of the Netherlands (2005)
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 (heart, pancreas, adrenals, kdiney, brain) Reduced biparietal, crown-rump length Reduced bw Reduced lung volume (not significant) Lung development affected (lung hypoplasia, reduced surface complexity of	Sekhon et al., 1999; via Health council of the Netherlands (2005)

Species + sex	Exposure route	Exposure duration	Dose or concentration	Effects observed	Reference
				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 abnormalities in fetuses resulting from mating in post-treatment week 1 and 2 of males treated for 20 weeks premating	Hemsworth 1981; via Health council of the Netherlands (2005)
Mouse (m)	intraperitoneal	15 d	0, 2, 4, 6 mg/kg bw/d	Reduced relative weight of testes, epididymis, seminal vesicle, prostate, and vas deferens. Reduced number of spermatocytes and spermatids	Reddy et al., 1998; via Health council of the Netherlands (2005)

B- human studies

Study subjects	Exposure route	Dose or concentration	Exposure duration	Effects observed	Reference
Non-smokers	inhalation	Single-breath inhalation of 0.1 mL of a nebulized nicotine solution (0, 1, 2, 4, 8, 16, 32 or 64 mg/mL)	Single-breath inhalation	Concentration-dependent cough response and broncho-constriction	Hansson et al., 1994
Non-smokers	inhalation	Single-breath inhalation of 0.01 mL of a	Repeated single-breath inhalations, 1	Dose-dependent increase in heart rate and systolic blood pressure	Hansson et al., 1994

Study subjects	Exposure route	Dose or concentration	Exposure duration	Effects observed	Reference
		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)	inhalation/15 sec, during total period of 5 min		
Not specified	intravenous	0,6 mg	-	Small to moderate increase in breathing frequency, heart rate, blood pressure; nausea	Ray 1991; via ACGIH 1994
Not specified	intravenous	3 mg	-	Increased blood pressure and heart rate in 8 volunteers; an initial slowing of the heart was produced in four volunteers; nausea	Henningfield et al., 1985; via ACGIH 1994

References appendix B:

ACGIH (1994). American Conference of Governmental Industrial Hygienist. Documentation of the Threshold Limit values and the biological Exposure Indices, 6th edition, april 1994.

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

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De zorg voor morgen begint vandaag