Container closure system for nicotine products

A worst-case health risk assessment of extractables

(30 mL/day exposure scenario)

V4

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EXECUTIVE SUMMARY

Chubby Gorilla manufactures container closure systems (CCSs). The CCSs are used to store nicotine-containing solutions e.g. e-liquids, which are subsequently "vaped" using appropriate (open tank system) electronic nicotine delivery systems (ENDS) and inhaled by consumers.

Chubby Gorilla has commissioned exhaustive extraction tests on a range of CCS components in order to identify/quantify extractables (i.e. potential leachables) that may migrate from the CCS into the e-liquid product. In actual use, the leachables could be inhaled by consumers.

Bibra was asked to consider a worst-case exposure scenario. Consequently, an extreme average daily consumption of 30 mL of e-liquid has been considered in this health risk assessment.

The analytical studies conducted on the PET containers (natural, solid black and translucent black), inner closures (natural and solid black), LDPE nozzle (natural and translucent black) and PP nozzle (natural and translucent black) components of the CCS detected a number of organic and inorganic extractables (potential leachables) to which consumers could potentially be exposed via vaping. From the results of the HS-GC-FID/MS, GC-FID/MS, LC-DAD/MS and ICP-MS analyses, worst-case consumer exposures were estimated, and possible health risks were assessed.

By comparing the highest anticipated concentration of these potential leachables within each inhalation with tolerable concentrations (TCs) of a potent respiratory tract irritant (formaldehyde), it was concluded that respiratory tract irritation is not of practical concern with respect to the identified extractable substances.

For chemically-identified extractables lacking mutagenic character (i.e. threshold toxins), the health risk assessment was based on key NOAELs from appropriate high-quality repeated dose toxicity studies where possible, supported by Expert Group derivations of tolerable exposure figures such as permitted daily exposures (PDEs), and health risk evaluations. Where necessary and appropriate, toxicity data on structurally-similar analogues were also used (in a read-across approach). ISO 10993-17 and ICH principles were followed, and the maximum worst-case exposures were compared with tolerable intakes (TIs) derived for each extractable.

Based on laboratory studies, Expert Group conclusions and/or Toxtree structure-activity relationships (SAR) the majority of the identified extractables were concluded to lack mutagenic potential and were thus assessed as threshold toxins. Margins of Safety were determined to be greater than unity for each extractable (or group of extractables), thus demonstrating tolerability.

As chromium (Cr) was identified in the ICP-MS analysis, the toxicological profiles of the most common valence states (Cr(III) and elemental chromium, Cr(0)) were assessed. The maximum

daily exposure to Cr was reassuringly >10-fold lower than the inhalation PDE derived for Cr by the ICH. Furthermore, as a highly health-precautionary measure, the toxicology of Cr(VI), a carcinogenic species, was also considered. By comparing the extreme worst-case estimates of exposure (assuming 100% of the extracted Cr to be in the hexavalent state) with tolerable intake values derived by Expert Groups, the resulting excess cancer risks were not determined to be within acceptable levels. However, tolerable cancer risks were estimated if up to 4% of the extracted Cr were in the hexavalent form. In reality, any Cr would likely be present in the zero-valence state, and any Cr(VI) present is likely to be rapidly reduced to Cr(III) and potential exposures to Cr(VI) are therefore likely to be far below 4% of the identified Cr. Overall, therefore, the potential exposure to Cr is unlikely to pose any significant health risks to consumers.

A respiratory sensitisation potential is acknowledged for both chromium and nickel. From the analysis, the calculated exposures were determined to be very low (0.3 µg/day and 0.7 ng/day, respectively). Although it is not possible to confidently determine a safe benchmark for these sensitisers, based on the very low estimates of exposure these two extractables are highly unlikely to pose any significant risk of inducting new cases of sensitisation. This is reassuring. However, it should be noted that the possibility of an occasional reaction in highly-sensitive individuals who have already been sensitised to these substances by other exposures, cannot be entirely excluded.

Overall it was concluded that the potential exposure to these extractables is unlikely to pose any significant health risks to consumers vaping e-liquid at 30 mL/day (a worst-case scenario).

Moreover, in reality, any potential leaching of these organic and inorganic species from the CCS into the e-liquid is likely to be far less extensive than has been observed in these exhaustive extractables studies (under exaggerated conditions). As such, far lower exposures would be anticipated in a leachables study and/or in real use than have been estimated in this extractables assessment, and thus even more reassuring Margins of Safety would be established.

BACKGROUND

Chubby Gorilla manufactures container closure systems (CCSs). The CCSs are used to store nicotine-containing solutions e.g. e-liquids, which are subsequently "vaped" using appropriate (open tank system) electronic nicotine delivery systems (ENDS) and inhaled by consumers.

Chubby Gorilla has commissioned exhaustive extraction tests on a range of CCS components in order to identify/quantify extractables (i.e. potential leachables) that may migrate from the CCS into the e-liquid product. In actual use, the leachables could be inhaled by consumers (if extracted by the e-liquid from the CCS).

Consequently, bibra was asked to conduct an independent worst-case health risk assessment of the extractables profile of the CCS components as a system.

EXPERTISE

Bibra was founded[1] in 1961 to provide independent, high-quality research, information and advice on chemical toxicology to industry and governmental departments and has a long-established record of objectivity and scientific excellence. All of the senior scientists in the current team (half of whom have worked together for 30-40+ years) are accredited and listed in the European (Eurotox) and UK Royal Society of Biology/British Toxicology Society Registers of Toxicologists and are thus bound by their specific codes of conduct.

Notably, in recent years, bibra has worked with a large number of companies in the e-cigarette, medical device and pharmaceutical sectors, assisting them with their evaluations of extractables and leachables.

TEST ITEMS

The CCS consists of the following product-contacting components: a PET[2] container, an HDPE[3] inner closure, and a PP[4] or LDPE[5] nozzle. The CCS also consists of a non-product-contacting component, a PP outer closure. This assessment focuses on the product-contacting components only (no extractables analyses were conducted on the PP outer closure).

The containers are available in a range of sizes (10 – 120 mL) and colours. This assessment is limited to the extractables identified in the natural (i.e. no colourant) and black (solid and translucent black) coloured components.

[1] As the British Industrial Biological Research Association.

[2] Polyethylene terephthalate.

[3] High density polyethylene.

[4] Polypropylene.

[5] Low density polyethylene.

Table 1. CCS combinations and product-contacting component weights

Container capacity	Mass (g)			
	Container	Inner Closure	Nozzle	
10 mL V3	2.97	1.11	0.49	LDPE (tall)
16.5 mL V3	3.57	1.11	0.49	
30 mL V3	5.39	1.11	0.49	
30 mL SC	6.97	2.31	0.87	PP (stubby)
50 mL V3	10.09	2.31	0.87	
60 mL V3	10.09	2.31	0.87	
60 mL Mini	9.54	3.39	1.05	PP (stubby)
60 mL SC	10.52	4.61	1.53	PP (stubby)
75 mL SC	12.20	4.61	1.53	
100 mL V3	13.47	4.61	1.53	
120 mL V3	14.86	4.61	1.53	

Table 2. Product-contacting component combinations considered in this report

Combination ID	PET Container	LDPE Nozzle	HDPE Inner Closure
1	Natural	Natural	Natural
2	Natural	Natural	Solid Black
3	Translucent black	Natural	Solid Black
4	Solid Black	Natural	Solid Black
5	Translucent black	Translucent black	Solid Black
Combination ID	**PET Container**	**PP Nozzle**	**HDPE Inner Closure**
6	Natural	Natural	Natural
7	Natural	Natural	Solid Black
8	Translucent black	Natural	Solid Black
9	Solid Black	Natural	Solid Black
10	Translucent black	Translucent black	Solid Black

ANALYTICAL METHODS

Determination of the extractables profiles was conducted by SGS Life Science Services. The results were supplied to bibra in two analytical reports (SGS, 2019a,b).

As part of this assessment to evaluate a 30-mL daily exposure, the following representative components were analysed by SGS:

- 120 mL PET Container (14.86 g) [natural, solid black and translucent black]
- 120 mL HDPE Inner Closure (4.61 g) [natural and black]
- 120 mL PP (stubby) Nozzle (1.53 g) [natural and translucent black]
- 30 mL LDPE (tall) Nozzle (0.49 g) [natural and translucent black].

The test samples were cut into small pieces and extracted in appropriate model solvents (pH 3 water: isopropanol, 20:80 v/v) at 50°C for approximately 72 hours. The extracts were then

analysed for volatile organic species, semi-volatile organic species, non-volatile organic species, and inorganic elements by HS-GC-FID/MS[6], GC-FID/MS[7], LC-DAD/MS[8] and ICP-MS[9] analyses, respectively (SGS, 2019a,b).

Appropriate blanks were also prepared and analysed.

CONSUMER EXPOSURE

Consumers vary widely in vaping habits. Bibra was asked to consider a worst-case high-end use exposure scenario in which consumers vape 30 mL e-liquid each day.

It was conservatively assumed that consumers might take 1000 inhalations ("vapes") of e-liquid vapour per day, and that each vape is approximately 100 mL in volume. If each vape lasts 10 seconds (covering inhalation and exhalation), this corresponds to a total 'peak' exposure time of approximately 3 hours per day. In reality, each vape will be separated by variable periods of inhalation of 'normal' air (i.e. without vape exposure). Hence it was assumed for the purposes of this risk assessment that 30 mL of e-liquid could be vaped within a 12-hour period each day.

It was considered that people could potentially be exposed to leachables in the e-liquid on many days in their lifetime (chronic exposure).

ANALYTICAL RESULTS AND EXPOSURE ESTIMATES

Analytical results were supplied in two laboratory reports (SGS, 2019a,b). The results were expressed as µg/sample for each analytical method. Chubby Gorilla provided bibra with the weight of each tested sample in order to convert these quantified results to µg/g values. These were then extrapolated to give µg/component values for each of the sizes of container, inner closure and appropriate nozzle. Only the maximum exposure estimates were taken forward to the risk assessment (resulting from the highest mass to capacity ratio).

The highest mass (g) to capacity (mL) ratio components were the 10 mL containers (2.97 g) and small inner closures (1.11 g); the 10 mL container is compatible with the small LDPE nozzle weighing 0.49 g. However, the PP nozzle is not compatible with the 10 mL containers. The highest mass to capacity ratio for the PP nozzle (0.87 g) therefore corresponds to its use with the 30 mL container (6.97 g) and the inner closure weighing 2.31 g. Consequently, exposure estimates were calculated by considering both the 10 mL CCS (with LDPE nozzle) and 30 mL CCS (with PP nozzle). Table 3 below presents the estimated exposures to the detected organic extractables from the GC-FID/MS and LC-DAD/MS analyses, expressed as averaged µg/day values. Table 4 below presents the estimated exposures to the inorganic extractables detected at above 1 ppb. No volatile organics were identified in the HS-GC/MS analyses of the subject components (SGS, 2019a,b).

[6] Headspace – gas chromatography – flame ionisation detector/mass spectrometry.

[7] Gas chromatography – flame ionisation detector/mass spectrometry.

[8] Liquid chromatography – diode array detector/mass spectrometry.

[9] Inductively coupled plasma/mass spectrometry.

In preparation of the sample extracts, entire components of the CCS were cut into pieces and submerged in an appropriate extraction solvent, as described above. However, in reality, the e-liquid is in contact with only inner surfaces of the components (containers, nozzles, and inner closures). Regardless of contact surface area in real-use conditions, it was assumed that the analytical results (µg/component) reflect actual exposure, and all extractables could be potential leachables during use of an electronic nicotine delivery device. It was also assumed that all detected extractables are present in the CCS components and are not by-products formed during extraction or analysis. These are conservative and worst-case assumptions.

Where these extractables were detected in several variants of the same component type, the highest figure has been taken forward to the risk assessment to evaluate the maximum worst-case exposure. Where these extractables were detected in different components of the CCS, the highest exposure values for each component type have been summed and taken forward to the risk assessment.

Table 3. Organic extractables and worst-case estimates of exposure for CCS with LDPE and PP nozzles

Identified organic extractable	Detection method	Component	Sample analysed			10 mL CCS (tall)			30 mL CCS (stubby)		
			Mass of sample (g)	Detected amount (µg/ sample)	Amount of extractable per gram (µg/g)	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/day) [10]	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/ day)[11]
2,4-Di-tert-butylphenol	GC-FID/MS	Inner closure, black	4.61	9.87	2.14	1.11	2.38	7.13	2.31	4.94	4.94
	LC-DAD/MS	Inner closure, black	4.61	28.86	6.26	1.11	6.95	20.85	2.31	14.46	14.46
	LC-DAD/MS	Inner closure, natural	4.61	10.03	2.18	1.11	2.42	7.25	2.31	5.03	5.03
	LC-DAD/MS	Nozzle (PP), natural	1.53	37.85	24.74	n/a[12]			0.87	21.52	21.52
	LC-DAD/MS	Nozzle (PP), translucent black	1.53	31.23	20.41	n/a			0.87	17.76	17.76
2,6-Di-tert-butylphenol[13]	GC-FID/MS	Inner closure, black	4.61	21.43	4.65	1.11	5.16	15.48	2.31	10.74	10.74

[10] Assuming daily intake of 30 mL e-liquid; filled from 10 mL CCS (with tall LDPE nozzle).

[11] Assuming daily intake of 30 mL e-liquid; filled from 30 mL CCS (with stubby PP nozzle).

[12] The PP nozzle is not compatible with the 10-mL containers.

[13] Identified as 2,6-bis(1,1-dimethylethyl)phenol.

Identified organic extractable	Detection method	Component	Sample analysed			10 mL CCS (tall)			30 mL CCS (stubby)		
			Mass of sample (g)	Detected amount (µg/ sample)	Amount of extractable per gram (µg/g)	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/day) [10]	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/ day)[11]
	GC-FID/MS	Nozzle (PP), natural	1.53	15.74	10.29	n/a			0.87	8.95	8.95
	GC-FID/MS	Nozzle (PP), translucent black	1.53	27.014	17.66	n/a			0.87	15.36	15.36
Dibutyl phthalate	GC-FID/MS	Inner closure, black	4.61	34.81	7.55	1.11	8.38	25.15	2.31	17.45	17.45
	GC-FID/MS	Inner closure, natural	4.61	37.30	8.09	1.11	8.98	26.94	2.31	18.69	18.69
	GC-FID/MS	Nozzle (PP), natural	1.53	32.18	21.03	n/a			0.87	18.30	18.30
	GC-FID/MS	Nozzle (PP), translucent black	1.53	40.46	26.44	n/a			0.87	23.00	23.00
Irgafos 168[14]	LC-DAD/MS	Inner closure, natural	4.61	103.86	22.53	1.11	25.01	75.02	2.31	52.04	52.04

[14] Also identified as 'phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)'.

Identified organic extractable	Detection method	Component	Sample analysed			10 mL CCS (tall)			30 mL CCS (stubby)		
			Mass of sample (g)	Detected amount (µg/ sample)	Amount of extractable per gram (µg/g)	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/day) [10]	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/ day)[11]
	GC-FID/MS	Inner closure, natural	4.61	62.343	13.52	1.11	15.01	45.03	2.31	31.24	31.24
Tris(2,4-di-tert-butylphenyl) phosphate[15] [Irgafos 168 oxidation product]	GC-FID/MS	Inner closure, natural	4.61	64.387	13.97	1.11	15.50	46.51	2.31	32.26	32.26
	LC-DAD/MS	Inner closure, natural	4.61	17.06	3.70	1.11	4.11	12.32	2.31	8.55	8.55
Irganox 1010	LC-DAD/MS	Inner closure, natural	4.61	11.72	2.54	1.11	2.82	8.47	2.31	5.87	5.87
Irganox 1076	LC-DAD/MS	Inner closure, natural	4.61	15.56	3.38	1.11	3.75	11.24	2.31	7.80	7.80
	GC-FID/MS	Inner closure, natural	4.61	32.617	7.08	1.11	7.85	23.56	2.31	16.34	16.34
n-Pentadecylcyclohexane	GC-FID/MS	Nozzle (LDPE), natural	0.49	6.792	13.86	0.49	6.79	20.38	n/a[16]		

[15] Also identified as tris(3,5-di-tert-butylphenyl) phosphate.

[16] The LDPE nozzle is not compatible with the 30-mL containers.

Identified organic extractable	Detection method	Component	Sample analysed			10 mL CCS (tall)			30 mL CCS (stubby)		
			Mass of sample (g)	Detected amount (µg/ sample)	Amount of extractable per gram (µg/g)	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/day) [10]	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/ day)[11]
Phthalic Acid Cyclic Oligomer	LC-DAD/MS	Container, natural	14.86	286.65	19.29	2.97	57.29	171.87	6.97	134.45	134.45
	LC-DAD/MS	Container, solid black	14.86	120.72	8.12	2.97	24.13	72.38	6.97	56.62	56.62
	LC-DAD/MS	Container, translucent black	14.86	362.57	24.40	2.97	72.47	217.40	6.97	170.06	170.06
C8-C20 acyclic alkanes											
5-Ethyl-2-methyl-octane	GC-FID/MS	Inner closure, black	4.61	9.025	1.96	1.11	2.17	6.52	2.31	4.52	4.52
2-Methyloctadecane	GC-FID/MS	Inner closure, black	4.61	8.326	1.81	1.11	2.00	6.01	2.31	4.17	4.17
Decane	GC-FID/MS	Inner closure, natural	4.61	31.214	6.77	1.11	7.52	22.55	2.31	15.64	15.64
Dodecane	GC-FID/MS	Inner closure, natural	4.61	85.315	18.51	1.11	20.54	61.63	2.31	42.75	42.75
	GC-FID/MS	Nozzle (LDPE), translucent black	0.49	11.89	24.27	0.49	11.89	35.67	n/a		

Identified organic extractable	Detection method	Component	Sample analysed			10 mL CCS (tall)			30 mL CCS (stubby)		
			Mass of sample (g)	Detected amount (µg/ sample)	Amount of extractable per gram (µg/g)	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/day) [10]	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/ day)[11]
Eicosane	GC-FID/MS	Inner closure, natural	4.61	10.106	2.19	1.11	2.43	7.30	2.31	5.06	5.06
Hexadecane	GC-FID/MS	Inner closure, natural	4.61	73.598	15.96	1.11	17.72	53.16	2.31	36.88	36.88
Octadecane	GC-FID/MS	Inner closure, natural	4.61	29.22	6.34	1.11	7.04	21.11	2.31	14.64	14.64
Pentadecane	GC-FID/MS	Inner closure, black	4.61	8.839	1.92	1.11	2.13	6.38	2.31	4.43	4.43
Tetradecane	GC-FID/MS	Inner closure, natural	4.61	101.843	22.09	1.11	24.52	73.57	2.31	51.03	51.03
Tridecane	GC-FID/MS	Nozzle (LDPE), natural	0.49	8.267	16.87	0.49	8.27	24.80	n/a		
	GC-FID/MS	Nozzle (LDPE), translucent black	0.49	50.02	102.1	0.49	50.02	150.1	n/a		
Undecane	GC-FID/MS	Nozzle (PP), natural	1.53	13.61	8.9	n/a			0.87	7.74	7.74

Identified organic extractable	Detection method	Component	Sample analysed			10 mL CCS (tall)			30 mL CCS (stubby)		
			Mass of sample (g)	Detected amount (µg/ sample)	Amount of extractable per gram (µg/g)	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/day) [10]	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/ day)[11]
	GC-FID/MS	Nozzle (LDPE), translucent black	0.49	13.175	26.89	0.49	13.18	39.53	n/a		
Fatty amides											
(Z)-9-Octadecenamide (oleamide)	GC-FID/MS	Nozzle (LDPE), natural	0.49	55.342	112.94	0.49	55.34	166.03	n/a		
	GC-FID/MS	Nozzle (LDPE), translucent black	0.49	62.155	126.85	0.49	62.16	186.47	n/a		
9-Octadecenamide	GC-FID/MS	Nozzle (LDPE), natural	0.49	3.148	6.42	0.49	3.15	9.44	n/a	n/a	n/a
(Z)-13-Docosenamide (erucamide)	GC-FID/MS	Inner closure, natural	4.61	10.296	2.23	1.11	2.48	7.44	2.31	5.16	5.16
	GC-FID/MS	Nozzle (LDPE), natural	0.49	2.837	5.79	0.49	2.84	8.51	n/a		
Hexadecanamide (palmitamide)	GC-FID/MS	Nozzle (LDPE), natural	0.49	6.792	13.86	0.49	6.79	20.38	n/a		

Identified organic extractable	Detection method	Component	Sample analysed			10 mL CCS (tall)			30 mL CCS (stubby)		
			Mass of sample (g)	Detected amount (µg/ sample)	Amount of extractable per gram (µg/g)	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/day) [10]	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/ day)[11]
Octadecanamide (stearamide)	GC-FID/MS	Nozzle (LDPE), natural	0.49	8.689	17.73	0.49	8.69	26.07	n/a		
Fatty acids											
n-Hexadecanoic acid	GC-FID/MS	Inner closure, black	4.61	34.818	7.55	1.11	8.38	25.15	2.31	17.45	17.45
	GC-FID/MS	Inner closure, natural	4.61	37.299	8.09	1.11	8.98	26.94	2.31	18.69	18.69
	GC-FID/MS	Nozzle (PP), natural	1.53	32.175	21.03	n/a			0.87	18.30	18.30
	GC-FID/MS	Nozzle (PP), translucent black	1.53	40.46	26.44	n/a			0.87	23.00	23.00
Octadecanoic acid	GC-FID/MS	Inner closure, black	4.61	18.802	4.08	1.11	4.53	13.58	2.31	9.42	9.42
	GC-FID/MS	Inner closure, natural	4.61	19.237	4.17	1.11	4.63	13.90	2.31	9.64	9.64

Identified organic extractable	Detection method	Component	Sample analysed			10 mL CCS (tall)			30 mL CCS (stubby)		
			Mass of sample (g)	Detected amount (µg/ sample)	Amount of extractable per gram (µg/g)	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/day) [10]	Mass of compatible component (g)	Amount of extractable per component (µg)	Exposure (µg/ day)[11]
	GC-FID/MS	Nozzle (PP), natural	1.53	14.472	9.46	n/a			0.87	8.23	8.23
	GC-FID/MS	Nozzle (PP), translucent black	1.53	22.032	14.4	n/a			0.87	12.53	12.53

Table 4. Inorganic elements and worst-case estimates of exposure

Identified element	Component	Sample analysed			10 mL CCS (tall)			30 mL CCS (stubby)		
		Mass of sample (g)	Detected amount (ng/mL)	Mass of element per gram (ng/g)[17]	Mass of compatible component (g)	Amount of extractable per component (ng)	Exposure (ng/day)[18]	Mass of compatible component (g)	Amount of extractable per component (ng)	Exposure (ng/day)[19]
Barium	Container (PET), natural	14.86	7.5	50.47	2.97	149.90	449.70	6.97	351.78	351.78
	Container (PET), translucent black	14.86	1.8	12.11	2.97	35.98	107.93	6.97	84.43	84.43
	Inner closure, natural	4.61	1.9	3.43	1.11	3.81	11.44	2.31	7.93	7.93
	Nozzle (PP), natural	1.53	1.2	9.15	n/a			0.87	7.96	7.96
	Nozzle (LDPE), natural	0.49	2.5	59.52	0.49	29.17	87.50	n/a		

[17] Calculated given that one container weighing 14.86 g was extracted in 100 mL; three inner closures weighing 4.6 g each were extracted in 25 mL; three stubby (PP) nozzles weighing 1.53 g were extracted in 35 mL; and three tall (LDPE) nozzles weighing 0.49 g were extracted in 35 mL.

[18] Assuming daily intake of 30 mL e-liquid; filled from 10 mL CCS (with tall LDPE nozzle).

[19] Assuming daily intake of 30 mL e-liquid; filled from 30 mL CCS (with stubby PP nozzle).

Identified element	Component	Sample analysed			10 mL CCS (tall)			30 mL CCS (stubby)		
		Mass of sample (g)	Detected amount (ng/mL)	Mass of element per gram (ng/g)[17]	Mass of compatible component (g)	Amount of extractable per component (ng)	Exposure (ng/day)[18]	Mass of compatible component (g)	Amount of extractable per component (ng)	Exposure (ng/day)[19]
Chromium	Container (PET), natural	14.86	1.1	7.40	2.97	21.99	65.96	6.97	51.59	51.59
	Container (PET), translucent black	14.86	3.8	25.57	2.97	75.95	227.85	6.97	178.24	178.24
	Nozzle (LDPE), translucent black	0.49	2.4	57.14	0.49	28.00	84.00	n/a		
Copper	Container (PET), natural	14.86	6.1	41.05	2.97	121.92	365.75	6.97	286.12	286.12
	Container (PET), translucent black	14.86	5.4	36.34	2.97	107.93	323.78	6.97	253.28	253.28
	Inner closure, black	4.61	2	3.62	1.11	4.01	12.04	2.31	8.35	8.35
	Inner closure, natural	4.61	25.8	46.64	1.11	51.77	155.30	2.31	107.73	107.73

Identified element	Component	Sample analysed			10 mL CCS (tall)			30 mL CCS (stubby)		
		Mass of sample (g)	Detected amount (ng/mL)	Mass of element per gram (ng/g)[17]	Mass of compatible component (g)	Amount of extractable per component (ng)	Exposure (ng/day)[18]	Mass of compatible component (g)	Amount of extractable per component (ng)	Exposure (ng/day)[19]
	Nozzle (PP), natural	1.53	5.3	40.41	n/a			0.87	35.16	35.16
	Nozzle (PP), translucent black	1.53	2.3	17.54	n/a			0.87	15.26	15.26
	Nozzle (LDPE), natural	0.49	6.8	161.90	0.49	79.33	238.00	n/a		
	Nozzle (LDPE), translucent black	0.49	1.9	45.24	0.49	22.17	66.50	n/a		
Lithium	Container (PET), natural	14.86	4.2	28.26	2.97	83.94	251.83	6.97	197.00	197.00
	Container (PET), translucent black	14.86	3.7	24.90	2.97	73.95	221.85	6.97	173.55	173.55
	Nozzle (LDPE), natural	0.49	2	47.62	0.49	23.33	70.00	n/a		

Identified element	Component	Sample analysed			10 mL CCS (tall)			30 mL CCS (stubby)		
		Mass of sample (g)	Detected amount (ng/mL)	Mass of element per gram (ng/g)[17]	Mass of compatible component (g)	Amount of extractable per component (ng)	Exposure (ng/day)[18]	Mass of compatible component (g)	Amount of extractable per component (ng)	Exposure (ng/day)[19]
	Nozzle (LDPE), translucent black	0.49	1	23.81	0.49	11.67	35.00	n/a		
Nickel	Container (PET), translucent black	14.86	10.2	68.64	2.97	203.86	611.59	6.97	478.43	478.43
	Inner closure, natural	4.61	11.8	21.33	1.11	23.68	71.03	2.31	49.27	49.27
	Nozzle (PP), natural	1.53	6.1	46.51	n/a			0.87	40.47	40.47
	Nozzle (PP), translucent black	1.53	2.1	16.01	n/a			0.87	13.93	13.93
	Nozzle (LDPE), translucent black	0.49	1.2	28.57	0.49	14.00	42.00	n/a		
Lead	Inner closure, natural	4.61	2.5	4.52	1.11	5.02	15.05	2.31	10.44	10.44

Identified element	Component	Sample analysed			10 mL CCS (tall)			30 mL CCS (stubby)		
		Mass of sample (g)	Detected amount (ng/mL)	Mass of element per gram (ng/g)[17]	Mass of compatible component (g)	Amount of extractable per component (ng)	Exposure (ng/day)[18]	Mass of compatible component (g)	Amount of extractable per component (ng)	Exposure (ng/day)[19]
Antimony	Inner closure, black	4.61	1.5	2.71	1.11	3.01	9.03	2.31	6.26	6.26
Selenium	Container (PET), black	14.86	1.1	7.40	2.97	21.99	65.96	6.97	51.59	51.59
	Container (PET), translucent black	14.86	4.9	32.97	2.97	97.93	293.80	6.97	229.83	229.83
	Nozzle (LDPE), translucent black	0.49	1.7	40.48	0.49	19.83	59.50	n/a		
Vanadium	Inner closure, black	4.61	2.5	4.52	1.11	5.02	15.05	2.31	10.44	10.44

TOXICOLOGICAL RISK ASSESSMENT

GENERAL COMMENTS AND STRATEGY

The analytical studies detected a number of organic extractables that could potentially migrate into the nicotine solutions and subsequently reach consumers by "vaping". It was assumed that leachables would not degrade during the vaping process, and 100% of the extractables detected could reach the consumer in the aerosol of e-liquid.

While lacking a formal classification, the CCSs could be thought of as being similar to medical devices or pharmaceutical CCSs. Therefore, this toxicological risk assessment (TRA) was carried out based on the methodologies and principles outlined in:

- International Standard ISO 10993-17: 2002. Biological evaluation of medical devices. Part 17: Establishment of allowable limits for leachable substances (ISO, 2002).
- Use of International Standard ISO 10993-1, "Biological evaluation of medical devices. Part 1: Evaluation and testing within a risk management process." Guidance for Industry and Food and Drug Administration Staff. Document issued on June 16, 2016 (US FDA, 2016).
- United States Pharmacopeia (USP) chapters <1663> and <1664> on the assessment of extractables/leachables associated with pharmaceutical packaging/delivery systems (USP, 2019a,b).
- European Chemicals Agency (ECHA) Guidance on information requirements and chemical safety assessment, Chapter R8: Characterisation of dose [concentration]-response for human health. Version 2.1. November 2012 (ECHA, 2012).
- Guidance from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH, 2017, 2018, 2019) on pharmaceutical impurities.
- ISO Technical Specification 21726. Biological evaluation of medical devices – application of the threshold of toxicological concern (TTC) for assessing biocompatibility of medical device constituents (ISO, 2019).

This TRA evaluated the critical systemic toxicology for each identified extractable detected from container, inner closure and nozzle product-contacting components of the CCS. The potential for the extractables to cause respiratory tract irritation is also considered below.

In any health risk assessment, a key initial step is to establish whether a substance has any mutagenic potential, since that will determine whether it is assessed either as a threshold toxin, or as a non-threshold compound, which as a mutagen or genotoxic carcinogen might be lacking a threshold in its dose-response.

Tolerable Intake (TI) and/or Tolerable Concentration (TC) values (as appropriate) were derived primarily based on ISO 10993-17 and ICH guidelines, as well as by considering other Expert Group evaluations, where possible. When ISO and ICH guidelines differed on the standard uncertainty factor (UF) to be applied to account for a particular aspect, the higher (more health-precautionary) value was selected. These TI values were then compared with the worst-case exposure estimates, and Margins of Safety (MOSs) were generated.

For assessing potential mutagenicity and carcinogenicity risk from untested compounds, this health risk assessment made "worst-case" assumptions about structure, and took into account standard TTC principles, as well as expert guidance (M7) from the ICH, the European

Medicines Agency (EMA), and ISO in respect of intermittent and less-than-lifetime exposure to DNA-reactive mutagens.

TOXICITY DATA SEARCHES[20]

Bibra has access to comprehensive sources of toxicity data, including the (REACH-approved) bibra TRACE database (see Appendix I for details), the TOXNET system of databases (see Appendix II), eChemPortal (see Appendix III), the ECHA Information on Chemicals database (see Appendix IV), PubMed (including Medline) and the Registry of Toxic Effects of Chemical Substances (RTECS). For the chemically-identified substances that have well-studied toxicology profiles[21], and for formaldehyde (not an identified extractable, but used to inform on respiratory irritation potential of extractables), this health risk assessment relied heavily on Expert Group reports and opinions. Data-deficient organic extractables (with no suitable read-across source data available) were considered by application of the TTC approach (see Appendix V). The data searches were conducted in August 2019.

POTENTIAL FOR RESPIRATORY TRACT IRRITATION

In this assessment, it is critical to evaluate the potential for the extractables to cause respiratory tract irritation, as well as systemic toxicity health risks to consumers. Ideally, TC values would be derived in addition to TI values (as described above), based on reliable inhalation data. However, high-quality inhalation studies are often rare in the toxicological literature for extractable substances.

Nevertheless, it is possible to address the potential for local toxicity by considering the concentrations of each extractable within the inhaled vapour, and comparing these with health-precautionary benchmarks established for potent respiratory irritants.

The highest daily exposure to a single extractable, or group of chemically-related extractables, was estimated to 481 µg/day for the C8-C20 category of acyclic alkanes. Assuming (as a worst-case) that a consumer vapes all 30 mL of e-liquid within a 12-hour time period, and that the consumer inhales on average about 1 m^3 air/hour, the total concentration of non-cyclic alkanes within the vapour can be approximated to 40 µg/m^3. The concentrations of all other extractables are lower than this value. As a worst-case approach, this concentration was assessed as if the extractables were potent respiratory tract irritants, equipotent with formaldehyde.

Formaldehyde is classified in Europe as corrosive to the skin, and its non-cancer effects primarily result from its ability to irritate the mucous membranes (particularly of the eyes and nose, with the lungs affected at higher concentrations) (ATSDR, 1999; OEHHA, 2014; SCOEL, 2016). The World Health Organisation (WHO) has established an air quality guideline (AQG) value of 100 µg/m^3 (expressed as a 30-minute average) for continuous lifetime exposure to formaldehyde. WHO concluded that this concentration is apparently "one order of magnitude lower than a presumed threshold for cytotoxic damage to the nasal mucosa", "represents an exposure level at which there is a negligible risk of upper respiratory tract

[20] Disclaimer: searches are valid and complete as of the date of searching. Bibra accepts no responsibility for the accuracy, completeness or sufficiency of any databases or searching platforms employed.

[21] Specifically dibutyl phthalate, ethylene glycol, and all inorganic elements.

cancer in humans" and "will also prevent long-term health effects, including cancer" (WHO, 2010).

This AQG value is reassuringly 2.5-fold higher than the maximum estimated concentration to any of the detected extractables (40 µg/m^3).

Based on these estimated exposures, it can therefore be confidently concluded that none of the detected extractables would pose a respiratory tract irritation concern of any practical concern to consumers, even if any were known to have irritating properties.

As such, the remainder of this TRA focuses on systemic toxicity health risks.

2,4-DITERTBUTYLPHENOL AND 2,6-DITERTBUTYLPHENOL

The GC-FID/MS and LC-DAD/MS analyses detected 2,4-di-tert-butylphenol (2,4-DTBP; CAS RN 96-76-4) in the inner closure (black and natural) and PP nozzle (natural and translucent black) components. In addition, the GC-FID/MS analyses detected 2,6-di-tert-butylphenol (2,6-DTBP; CAS RN 128-39-2) in the inner closure (black) and PP nozzle (natural and translucent black) components (only used for the 30-mL device). Based on their structural similarities, the toxicological profiles of these two positional isomers are not expected to be significantly different, hence it is appropriate to assess them together at a combined exposure (a worst-case approach). The highest potential combined exposure is for consumers vaping 30 mL e-liquid per day by filling up their ENDS using the 30 mL CCS (with black inner closure and translucent black PP nozzle), who may be exposed to 2,4- and 2,6-DTBP at up to 58.32 µg/day, equivalent to approximately 0.97 µg/kg bw/day for a 60-kg individual.

Genotoxicity

2,6-DTBP did not provide any evidence of mutagenic potential in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, with or without a metabolic activation fraction (S9) derived from rat liver. Additionally, 2,6-DTBP was also inactive in *Escherichia coli* with and without S9 and did not induce chromosomal aberrations in Chinese hamster V79 lung cells with and without activation (US EPA, 2009). According to the results of an *in vitro* mammalian HGPRT assay summarised in the REACH dossier, 2,6-DTBP did not induce gene mutations in Chinese hamster V79 lung cells, with or without S9 (Afton Chemical S.P.R.L. (Woluwe) *et al.*, 2019).

Industry has also submitted a REACH dossier on 2,4-DTBP that summarises various genotoxicity studies. 2,4-DTBP was determined to be non-mutagenic in a bacterial reverse mutation [Ames] assay using *S. typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535, when tested at up to the cytotoxic limit (1500 µg/plate) with and without S9 in two separate experiments. A lack of mutagenic activity was also observed in a further two Ames tests with Salmonella strains TA1535, TA1537, TA98, TA100 and TA1538 [as well as *E. coli* WP2 uvrA (MHLW, undated)]. There was a treatment-related increased incidence of chromosome aberrations in Chinese hamster lung (CHL) fibroblasts when tested in the presence of S9 (MHLW, undated). However, administration of 800-1000 mg/kg bw/day for 2 days by oral gavage to male and female rats (5/sex/group) did not induce micronuclei in the bone marrow cells (BASF Lampertheim GmbH *et al.*, 2019).

Experts from the US Environment Protection Agency were satisfied that 2,6-DTBP did not show evidence of genotoxic potential, and consequently predicted 2,4-DTBP to be non-mutagenic (US EPA, 2009).

Overall, it was considered appropriate to assess 2,4- and 2,6-DTBP as non-mutagenic threshold toxins.

Sensitisation

In a maximization study compliant with OECD Test Guideline 406[22], 2,4-DTBP was non-sensitising to guinea pigs (10/sex) dermally challenged (24-hour patch) with the test compound at a concentration of 25% v/v following a two-stage induction with a 5% v/v concentration by intradermal injection and 10% v/v by topical application (48-hour patch) (BASF Lampertheim GmbH *et al.*, 2019).

Similarly, 2,6-DTBP failed to elicit a delayed hypersensitivity response in guinea pigs (10/sex) in another maximization test conducted according to OECD Test Guideline 406 (Afton Chemical S.P.R.L. (Woluwe) *et al.*, 2019).

ADME considerations

No substance-specific data were identified.

However, the REACH registrants for both substances have indicated that oral absorption is significant. The REACH registrants for 2,6-DTBP stated that the mean fractional absorption of the compound was predicted to be 0.89 (based on Simcyp v.10.0 computer modelling), and significant first pass effects were not expected (Afton Chemical S.P.R.L. (Woluwe) *et al.*, 2019). The REACH dossier on 2,4-DTBP evidently utilised an oral absorption value of 100% in extrapolating oral data to the inhalation route of exposure. This figure was based on the physico-chemical properties of the substance (low molecular weight, low water solubility and high partition coefficient) as well as a study on Tebufelone, a member of the chemical class of DTBP antirheumatic agents, indicating complete oral absorption (BASF Lampertheim GmbH *et al.*, 2019).

Repeated dose threshold toxicity

No substance-specific inhalation toxicity studies were identified.

In a repeated dose oral toxicity study identified as key by the REACH registrants for 2,4-DTBP[23], rats (15/sex/group) received 2,4-DTBP in the diet at dose levels of 0, 50, 150 or 300 mg/kg bw/day for around 6-9 weeks[24]; offspring (20/sex/group) were similarly treated

[22] Skin sensitisation.

[23] Although not an OECD guideline study, the experiment combines elements of a One-Generation Reproduction Toxicity Study (OECD 415) and the Repeated Dose 90-day Oral Toxicity Study in Rodents (OECD 408) and the REACH registrants considered the study to be relevant to address these endpoints. "In terms of repeat[ed] dose toxicity, it presents a worst-case study as the [F1] animals were exposed to the test substance during gestation, lactation and for 13 weeks following weaning" (BASF Lampertheim GmbH *et al.*, 2019). The assessment of the parental generation evidently focused on reproductive toxicity, rather than general systemic toxicity.

[24] In addition to a 28-day premating exposure period, females were exposed throughout mating, gestation and lactation (around 9 weeks in total) while males were exposed during mating until conception was established in females (around 6 weeks in total).

for 13 weeks[25], with a 4-week non-treatment period to assess reversibility. An extensive gross and histopathological[26] assessment was conducted on F1 animals. The critical effect was reduced growth in offspring exposed at the two highest dose levels, though this was only considered a "primary toxic effect" at the top dose[27]. Although liver weights were significantly increased at 300 mg/kg bw/day, these had normalised during the recovery period and were considered adaptive in nature in the light of the lack of accompanying histopathological effects. Kidney and spleen weight changes were also considered incidental in the absence of microscopic findings. The study no-observed-adverse-effect level (NOAEL) (F1; subchronic) was established as 150 mg/kg bw/day. Parental animals displayed "retarded growth" at 150 mg/kg bw/day and body weight losses at the top dose; no gross lesions were apparent. The F0 NOAEL (6-9 weeks) was also set at 150 mg/kg bw/day (BASF Lampertheim GmbH *et al.*, 2019).

A subsequent investigation in rats (6-12/sex/group), conducted to the relevant Japanese test guideline, involved the gavage administration of 2,4-DTBP at 0, 5, 20, 75 or 300 mg/kg bw/day for 28 days, followed by a 2-week recovery period. The principal effects were hepatic hypertrophy (with concomitant increased liver weight) and renal histopathology at the top dose, which showed a tendency towards reversal during the recovery period. The study NOAEL (subacute) was 75 mg/kg bw/day (BASF Lampertheim GmbH *et al.*, 2019).

In a related study, new-born rats (12/sex/group) received 2,4-DTBP by gavage at 0, 5, 40 or 300 mg/kg bw/day on postnatal day (PND) 4-21, prior to a non-treatment period of 9 weeks. Liver and kidney weight changes, accompanied by histopathological changes (persisting only in the kidney after the recovery period), were limited to the top dose group. The NOAEL for new-born rats was evidently 40 mg/kg bw/day (BASF Lampertheim GmbH *et al.*, 2019).

In a guideline-compliant (OECD TG 407[28]) study, rats (5/sex/group) were gavaged at 0, 15, 100 or 600 mg/kg bw/day for 28 days with 2,6-DTBP before being subjected to an extensive toxicity assessment. Effects on the kidney (increased weight associated with microscopic effects in males only) and various clinical chemistry parameters were observed in animals exposed at 600 mg/kg bw/day. Increases in liver weight (accompanied by hypertrophy) and caecum size were evident in the mid- and high-dose groups, though these findings were not considered to be of toxicological significance. The study NOAEL (subacute) was established as 100 mg/kg bw/day (Afton Chemical S.P.R.L. (Woluwe) *et al.*, 2019), in agreement with the US EPA conclusion (US EPA, 2009)[29].

The REACH registrants also described a subchronic toxicity study, compliant with OECD Test Guideline 408, in which rats (10/sex/group) were fed diets containing 2,6-DTBP at 0, 150, 500, 1600 or 4000 ppm for approximately 90 days. Slightly lower body weights, body weight gain and food consumption were identified at the highest tested dose, however, the animals recovered and these effects had reversed after a 28-day rest period. In addition, liver weight increases in the absence of any histopathological effects were considered to be adaptive

[25] Animals were exposed indirectly during the gestation and lactation periods as well as directly for 13 weeks after weaning.

[26] Limited to the control and high dose animals.

[27] At 150 mg/kg bw/day, the growth effects were deemed solely to be a consequence of reduced diet palatability.

[28] Repeated Dose 28-day Oral Toxicity Study in Rodents.

[29] The data submitters considered 15 mg/kg bw/day to be the study NOAEL (Schenectady International, undated). Evidently, the US EPA did not consider the observed effects on liver weight and caecum size to be critical.

changes of no toxicological significance, and had reversed during the 28-day rest period. Consequently, the study NOAEL was considered to be the highest tested dose, reportedly equivalent to 270 and 298 mg/kg bw/day for male and female rats, respectively (Afton Chemical S.P.R.L. (Woluwe) *et al*., 2019).

Health risk assessment

2,4- and 2,6-DTBP displayed a consistent lack of mutagenic activity *in vitro*. Although the 2,4-isomer (alone) gave some indication of clastogenicity in mammalian cells in culture, this is overruled by the reassuring *in vivo* mouse micronucleus test result on the same substance. Overall, the substances are not considered to be genotoxic.

No adequate inhalation studies (for TI derivation) were identified, but several repeated dose oral toxicity studies are suitable for this purpose.

Administration of 2,4-DTBP to rats generally resulted in growth suppression; effects on the liver and kidney were also observed, though these were not considered to be toxicologically relevant in the various investigations. The 2,4-DTBP REACH registrants utilised a NOAEL of 150 mg/kg bw/day (based on a significant adverse effect on growth at the highest tested dose of 300 mg/kg bw/day) identified in a high-quality 13-week dietary study in rats as the basis for derived-no-effect-level (DNEL[30]) calculations. However, effects on growth were also seen at the mid-dose (150 mg/kg bw/day) and, consequently, this value might be better thought of as a mild lowest-observed-adverse-effect level (LOAEL). The substance-specific subchronic rodent study (taking 50 mg/kg bw/day as a health-precautionary NOAEL) is the most conservative point of departure (PoD) for the derivation of a TI. In support[31], the US EPA (2009) considered NOAEL and LOAEL values of 100 and 600 mg/kg bw/day from a 4-week gavage study on 2,6-DTBP to be applicable to 2,4-DTBP. Moreover, a higher NOAEL of 270-298 mg/kg bw/day was identified in a 90-day oral toxicity study with 2,6-DTBP.

TI = NOAEL/MF

MF[32] = UF1 x UF2 x UF3

UF1 = 10 for inter-individual variation among humans.

UF2 = 10 for extrapolation from data derived in a species other than humans. [The default factor to extrapolate from data derived in rats is 5 according to ICH (2018).]

UF3 = 10. A health-precautionary NOAEL from a good-quality subchronic oral toxicity study with 2,4-DTBP is used as the key PoD. ICH (2018) guidelines recommend a factor of 5 is appropriate to account for differences in study duration when data are derived from subchronic rodent studies. This factor is therefore applied here. Many supporting studies exist for DTBP isomers and there is a high degree of confidence in the PoD. With regards to extrapolation from an oral route to an inhalation exposure, no relevant bioavailability data

[30] A DNEL is the level of exposure to the substance above which humans should not be exposed. Health risks are considered to be adequately controlled if exposures are kept below the DNELs. These values represent the views of the submitting consortium. In general, the amount of information disseminated on the ECHA website is insufficient for easy or independent verification of these DNELs.

[31] Using ICH default modifying factors (MFs) for study duration, a 28-day NOAEL of 100 mg/kg bw/day is equivalent to a 13-week NOAEL of 50 mg/kg bw/day

[32] Modifying factor.

were identified. Although the REACH registrants have suggested that gastrointestinal absorption is significant (and first-pass effects are minimal), ECHA (2012) guidance recommends a default absorption factor of 50% in the absence of any other information. A factor of 2 is therefore applied here as an approximate factor to account for cross-route extrapolation.

TI = NOAEL/MF = 50 mg/kg bw/day/(10 x 10 x 10) = 0.05 mg/kg bw/day.

Consumer population	TI for 2,4- and 2,6-DTBP (µg/kg bw/day)	Maximum exposure to 2,4- and 2,6-DTBP (µg/kg bw/day)	Margin of Safety
Adults	50	0.97	51.5

As shown in the table above, the maximum combined exposure to 2,4- and 2,6-DTBP resulting from vaping 30 mL e-liquid per day is 51.5-fold lower than the highly conservative TI. It can therefore be confidently concluded that exposure to these two structurally related substances is highly unlikely to pose any significant health risk to consumers.

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DIBUTYL PHTHALATE

The GC-FID/MS analyses detected di-n-butyl phthalate (DBP; CAS RN 84-74-2) in the (natural and black) inner closures and in the PP nozzle (natural and translucent black) (only used for the 30-mL device). Consumers vaping 30 mL e-liquid per day by filling up their ENDS using the 30 mL CCS (with natural inner closure and translucent black PP nozzle) may be exposed to DBP at up to 41.7 µg/day, equivalent to approximately 0.69 µg/kg bw/day for a 60-kg individual.

Genotoxicity

Numerous Expert Groups consider DBP [as well as related phthalates] to lack mutagenic potential. These phthalates have been extensively tested for genotoxic properties (Danish EPA, 2015; ECB, 2004; ECHA, 2012; EFSA, 2005, 2019; EMA, 2014). As such, it is considered appropriate to assess DBP as a threshold toxin.

Sensitisation

DBP is not considered to be a skin sensitiser (ECB, 2004).

ADME considerations

Measurements in rodents exposed orally to low doses of phthalates indicate that gastrointestinal absorption is rapid and that absorbed levels are close to 100% for DBP (EFSA, 2019). In its risk assessment of DBP, ECHA applied oral, dermal and inhalation absorption factors of 100, 10 and 100%, respectively (ECHA, 2012).

Repeated dose threshold toxicity

A number of phthalates including di(2-ethylhexyl) phthalate (DEHP) and DBP have come under intense regulatory scrutiny in recent years because of concern over their endocrine disrupting properties and their potential reproductive and developmental effects in humans. As different phthalates may cause common health effects, it has been proposed that the cumulative risk from their combined exposures could be considered (ECCC/HC, 2017).

The European Food Safety Authority has considered the health risks posed by dietary phthalates. For DBP, a dietary lowest-observed-adverse-effect concentration (LOAEC) of 20 mg/kg diet (equivalent to 1.5-3 mg/kg bw/day) was identified, based on reduced spermatocyte development and effects on the mammary gland that were identified in a developmental toxicity study in rats. By applying a UF of 200[33] to a LOAEL of 2 mg/kg bw/day, an oral tolerable daily intake (TDI) of 0.01 mg/kg bw was proposed (EFSA, 2019).

The Californian Office of Environmental Health Hazard Assessment (OEHHA) identified the same key study as EFSA (2005, 2019) to characterise the reproductive toxicity hazard to DBP, taking the LOAEL as 1.5 mg/kg bw/day. They divided this by a factor of 10,000[34] to calculate an intake (0.15 µg/kg bw/day) upon which to base a maximum allowable dose level (MADL) in the drinking water (8.7 µg/day, based on a body weight of 58 kg). It was noted that as DBP is

[33] A factor of 2 to convert the LOAEL to a NOAEL, and 10 and 10 for inter- and intra-species differences.

[34] The LOAEL of 1.5 mg/kg bw/day was divided by factors of 10 to account for extrapolation of the LOAEL to a NOAEL, to account for the severity of the effects, and to account for inter- and intra-species differences.

nearly completely absorbed following oral administration, the MADL can be considered as an "absorbed dose" (OEHHA, 2007).

In a guideline of the European Medicines Agency, a Permitted Daily Exposure (PDE) of 0.01 mg/kg bw/day was derived for DBP, again on the basis of adverse reproductive effects in rats. The PDE is applicable to all exposure routes (EMA, 2014). ECHA has reported a slightly lower oral DNEL of 6.7 µg/kg bw/day for DBP based on a developmental toxicity study in rats (ECHA, 2016), maintaining a previous derivation (ECHA, 2012).

Health risk assessment

The most conservative of the daily intake figures derived by an Expert Group is the MADL of 8.7 µg/day, equivalent to 0.15 µg/kg bw/day for a 58-kg woman, derived by the OEHHA (2007). However, the 10,000 UF applied in the derivation seems excessive and was not adopted in more recent EFSA and EMA reviews. It is therefore appropriate to select the EFSA TDI of 0.01 mg/kg bw/day (which matches the EMA PDE exactly) as an appropriate oral TI for DBP. OEHHA noted that the oral MADL could also be considered to represent a systemic (absorbed dose), suggesting that route-route extrapolation is unnecessary; absorption following inhalation is assumed to be 100%.

Consumer population	TI for DBP (µg/kg bw/day)	Maximum exposure to DBP (µg/kg bw/day)	Margin of Safety
Adults	10	0.69	14.5

As shown in the table above, the potential exposure to DBP resulting from vaping 30 mL e-liquid per day is considerably lower than the highly conservative TI, indicating tolerability.

It can therefore be confidently concluded that exposure to DBP is unlikely to pose any significant health risk to consumers.

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IRGAFOS 168 AND OXIDATION PRODUCT

The GC-FID/MS and LC-DAD/MS analyses detected Irgafos 168 (CAS RN 31570-04-4) in the inner closure (natural) components at a maximum of 22.53 µg/g. Consumers vaping 30 mL e-liquid per day may therefore be exposed at up to 75 µg/day, equivalent to approximately 1.25 µg/kg bw/day for a 60-kg individual.

In addition, the oxidation product of Irgafos 168, tris(2,4-di-tert-butylphenyl) phosphate (CAS RN 95906-11-9) was identified in the GC-FID/MS and LC-DAD/MS analyses in the inner closure (natural) components at a maximum of 13.97 µg/g. Consumers vaping 30 mL e-liquid per day may therefore be exposed at up to 46.5 µg/day, equivalent to approximately 0.78 µg/kg bw/day.

The only structural difference between the oxidised and unoxidised form is that the 2,4-di-tert-butylphenyl groups are bonded to a central phosphate group, rather than a central phosphite group, in the oxidation product. Moreover, there is evidence that Irgafos 168 is oxidised in the gastrointestinal tract of rats (OECD, 2009). Given this, and their high structural similarity, it seems appropriate to assess the two chemicals together. There is also expert support for grouping the molecules; EFSA did similarly in its evaluation of food-contact material substance No 97 [FCM 97]; a mixture of 4-(1,1-dimethylpropyl)phenyl- and 2,4-bis(1,1-dimethylpropyl)phenyl- phosphate/phosphite triesters (CAS RN 939402-05) (EFSA, 2017), which have very similar structures to those being assessed here. As toxicity data on oxidised Irgafos 168 are lacking, information on oxidised FCM 97 (FCM 97 phosphate) are also considered as read-across in this risk assessment. The maximum combined exposure to oxidised/unoxidised Irgafos 168 is therefore 121.5 µg/day, equivalent to 2.0 µg/kg bw/day for a 60-kg individual.

Genotoxicity

A US EPA review and the REACH dossier on Irgafos 168 summarise studies reporting that the chemical did not induce chromosome aberrations or micronuclei in the bone marrow of hamsters given oral doses of up to 2000 mg/kg bw/day for 2 days, or in the spermatocytes of mice given up to 4444 mg/kg bw by intraperitoneal injection (on 5 consecutive days) or oral gavage (on 5 out of 10 days). No evidence of a dominant lethal mutation effect was found when male mice were treated with up to 3000 mg/kg bw on a single occasion by gavage and then mated with untreated females, each week for 6 consecutive weeks. Irgafos 168 also gave reassuring results in tests for bacterial (Ames) and yeast mutations, and no evidence of carcinogenicity was seen when rats (70/sex/dose level) were fed Irgafos 168 at up to 2000 ppm in the diet (equivalent to 58-147 mg/kg bw/day) for 2 years (Addivant UK Ltd (JVAS) *et al.*, 2019; US EPA, 2001).

Although no test data were identified for oxidised Irgafos 168, *in vitro* tests on FCM 97 phosphate have been performed in accordance with GLP and the relevant OECD Test Guidelines. There was no evidence of mutagenicity in *S. typhimurium* strains TA98, TA100, TA1535 or TA1537 or in *E. coli* strain WP2 *uvr*A, with or without S9. Findings were similarly reassuring in two mouse lymphoma (L5178Y) cell gene mutation assays. Furthermore, the substance did not induce chromosomal aberrations in human lymphocytes in two separate *in vitro* tests (EFSA, 2017).

The data indicate that oxidised and unoxidised Irgafos 168 lack mutagenic potential, a conclusion supported by the fact that the unoxidised form has been allocated a TDI by the Scientific Committee on Food (see below). It is therefore appropriate to assess the mixture as a threshold toxin.

Sensitisation

Industry's REACH dossier indicates that Irgafos 168 did not produce skin sensitisation effects in guinea pigs in a study conducted in accordance with OECD Test Guideline 406 (Addivant UK Ltd (JVAS) *et al.*, 2019).

Moreover, no structural alerts for skin sensitisation reactivity domains were identified in Toxtree (version 3.1.0, with plug-ins) for the oxidation product. The phosphate group is not associated with sensitisation potential.

As such, the potential exposure to these related substances is unlikely to produce sensitisation effects.

ADME considerations

Uptake of Irgafos 168 from the gastrointestinal tract is "extremely limited" (OECD, 2009). After the oral dosing [presumably by gavage] of rats with radio-labelled Irgafos 168 at 0.26 or 5.3 mg/kg bw, the test material was excreted mainly in the faeces (95-97% of the applied radioactivity) within 72 hours. Less than 0.4% was recovered in the urine during the 72-hour observation period, and blood radioactivity and residual radioactivity in the body were <0.05% of dose. A University dissertation noted that, after rats were administered Irgafos 168 by oral gavage, the major metabolite identified in the faeces (collected over a 5-day period) was oxidised Irgafos 168 (77% of the ingested dose). Overall, the data support the view that direct oxidation of Irgafos 168 occurs in the gastrointestinal tract, and the oxidised Irgafos 168 formed is largely unabsorbed and eliminated in the faeces (OECD, 2009).

Although no data were identified on absorption following inhalation, it seems that absorption through the lungs is also likely to be low. Presumably, deposition in the respiratory tract would result in clearance by the mucociliary elevator and subsequent swallowing i.e. leading ultimately to an oral exposure.

Repeated dose threshold toxicity

Several oral toxicity studies in rats are available for Irgafos 168 (including 90-day and 2-year investigations). The EU SCF has derived an oral TDI of 1 mg/kg bw for Irgafos 168 from the long-term toxicity and carcinogenicity data in rats, in which a systemic NOAEL of 58-147 mg/kg bw/day (the highest tested dose level) was identified (SCF, 1999). In a key subchronic toxicity study, there were no adverse effects in rats (20/sex/group) given up to 500 mg/kg bw/day for 13 weeks by gavage. Survival, clinical appearance, growth, urine composition, blood chemistry, the eyes and the gross and microscopic appearance of [unspecified] organs and tissues were monitored. The only changes seen were increased kidney and thyroid weights, without histopathological change. Reassuring results were obtained in a two-generation reproduction study in rats exposed via the diet (total study time 18 weeks) and in a study of developmental toxicity potential involving gavage administration to rabbits on days 6-18 of pregnancy (US EPA, 2001). No evidence of toxicity was seen in rats receiving Irgafos 168 in the diet at concentrations supplying up to about 58-147 mg/kg bw/day for 2 years (Addivant UK Ltd (JVAS) *et al.*, 2019).

No long-term toxicity data were identified on oxidised Irgafos 168, however a 91-day study, compliant with OECD Test Guideline 408[35] has been conducted on FCM 97 phosphate. Rats received 0, 100, 300 or 1000 mg/kg bw/day by oral gavage. The control and high-dose group were also given a recovery period without treatment for 28 days. The study NOAEL was 300 mg/kg bw/day, based on effects on the blood (increased cholesterol in females and mild prolongations of activated partial thromboplastin time in both sexes) in the high-dose group (EFSA, 2017).

[35] Repeated Dose 90-Day Oral Toxicity Study in Rodents.

Health risk assessment

No inhalation studies with Irgafos 168 or its oxidation product were identified, but several repeated dose oral toxicity studies are suitable for TI derivation.

The key subchronic study NOAEL of 500 mg/kg bw/day provides the most reliable PoD from which to derive a TI for Irgafos 168 as well as its oxidation product.

TI = NOAEL/MF

MF = UF1 x UF2 x UF3

UF1 = 10 for inter-individual variation among humans.

UF2 = 10 for extrapolation from data derived in a species other than humans. [The default factor to extrapolate from data derived in rats is 5 according to ICH (2018).]

UF 3 = 500. A NOAEL from a good-quality subchronic oral toxicity study with Irgafos 168 is used as the key PoD. ICH (2018) guidelines recommend a factor of 5 is appropriate to account for differences in study duration when data are derived from subchronic rodent studies. This factor is therefore applied here. According to ICH guidance, in the absence of data for the inhalation route of administration, MFs based on oral bioavailability may be used to derive tolerable intake levels. Irgafos 168 is poorly absorbed following oral administration to rats (>90% of an ingested dose was recovered in the faeces after 72 hours, and the extent of possible biliary excretion and first-pass metabolism is unknown). As such, a conservative factor of 100 was used to account for the cross-route extrapolation. This is likely to be very health-precautionary, as the compounds are likely to be poorly absorbed following inhalation and eventually probably swallowed after transport along the mucociliary elevator. There is a high degree of confidence on the current dataset for Irgafos 168, and there are several supporting studies. No additional factor for read-across was considered necessary for the oxidation product given its very high structural similarity to Irgafos 168, and both chemicals were evaluated together at a total dose of 0.28 µg/kg bw/day.

TI = NOAEL/MF = 500 mg/kg bw/day/(10 x 10 x 500) = 0.01 mg/kg bw/day.

Consumer population	TI for Irgafos 168 (µg/kg bw/day)	Maximum exposure to oxidised/unoxidised Irgafos 168 (µg/kg bw/day)	Margin of Safety
Adults	10	2.0	5

As shown in the table above, the maximum combined exposure to Irgafos 168 and its oxidation product resulting from vaping 30 mL e-liquid per day is 5-fold lower than the TI. It can therefore be confidently concluded that exposure to these two structurally related extractables is highly unlikely to pose any significant health risk to consumers.

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IRGANOX 1010

The LC-DAD/MS analyses detected Irganox 1010 (CAS RN 6683-19-8) in the inner closure (natural) component at 2.54 µg/g. Consumers vaping 30 mL e-liquid per day may therefore be exposed at up to 8.47 µg/day, equivalent to approximately 0.14 µg/kg bw/day for a 60-kg individual.

Genotoxicity

Irganox 1010 was not mutagenic in Ames tests using four or five strains of Salmonella, both in the presence and absence of S9, nor in an *in vivo* dominant lethal test (equivalent or similar to OECD Test Guideline 478[36]) in mice given gavage doses of up to 3000 mg/kg bw. It did not induce micronuclei (presumably in the bone marrow) in rats given a single oral dose of 5000 mg/kg bw, and neither micronuclei nor chromosome aberrations were induced in the bone marrow of hamsters receiving up to 2000 mg/kg bw/day on 2 days by gavage. There was no evidence of carcinogenicity in these animals, or in mice given up to 1000 ppm in the diet for 2 years [about 130 mg/kg bw/day] (3M Belgium BVBA/SPRL *et al.*, 2019; CIR, 2014). These data indicate that it is appropriate to assess Irganox 1010 as lacking mutagenic potential, a conclusion supported by the fact that it has been allocated a TDI figure by the SCF (see below).

[36] Genetic Toxicology: Rodent Dominant Lethal Test.

Sensitisation

Irganox 1010 at a concentration of 0.5% did not induce sensitisation in a HRIPT[37] in 50 subjects. Moreover, the substance was also non-sensitising in a Maurer optimization test. Guinea pigs (ten/sex) were administered ten intradermal injections over a 3-week period, followed by repeated topical applications over a second 3-week period. During the challenge phase, Irganox 1010 (0.1% in propylene glycol) was administered to the animals, either by topical application or intradermal injection, 14 and 24 days after the last induction (CIR, 2014). As such, it is considered unlikely that exposure to this substance would induce any sensitisation effects in consumers.

ADME considerations

The REACH registrants considered that only trace amounts of ingested Irganox 1010 are absorbed from the gastrointestinal tract, based on its physicochemical properties. In one limited oral absorption study briefly summarised in the REACH dossier, two rats were gavaged with a single dose of radiolabelled Irganox 1010 and the blood, gastrointestinal tract, urine, faeces, cage washings expired air and carcass were analysed for radioactivity. According to the REACH registrants, the data indicated that about 2-3% of Irganox 1010 was absorbed from the gastrointestinal tract, with 80-84% being excreted in the faeces and urine (3M Belgium BVBA/SPRL *et al.*, 2019).

Repeated dose threshold toxicity

A review of the safety of Irganox 1010, conducted by an Expert Panel for the US Cosmetic Ingredient Review (CIR), described how no adverse effects were seen in dogs fed up to 10,000 ppm in the diet for 3 months [about 250 mg/kg bw/day]. In rats (50/sex/group) exposed for 2 years, the dietary NOAEC was 3000 ppm (135 mg/kg bw/day in males; 166 mg/kg bw/day in females), based on minimal effects on growth, food consumption and thyroid weight. There was no evidence of carcinogenicity in these animals, or in mice given up to 1000 ppm in the diet for 2 years [about 130 mg/kg bw/day]. No evidence of reproductive or developmental toxicity was seen in a 2-generation study on rats fed diets containing up to 10,000 ppm [about 500 mg/kg bw/day], or in prenatal studies on rats and mice given up to 1000 mg/kg bw/day on days 6-15 of gestation by gavage (3M Belgium BVBA/SPRL *et al*., 2019; CIR, 2014).

Although it did not specifically describe them, SCF noted the existence of (probably at least some of the same) subacute/subchronic studies in rats and dogs, chronic studies in rodents, and reproductive/developmental data. The Committee used these to derive an oral TDI for Irganox 1010 of 3 mg/kg bw (SCF, 1995).

Health risk assessment

No inhalation studies with Irganox 1010 were identified, but several repeated dose oral toxicity studies are suitable for TI derivation.

[37] Human repeated insult patch test. These generally involve treatment with the test substance as nine 24- or 48-hour dermal applications over 3 weeks, followed (after a 2-week period without treatment) by a 24- or 48-hour challenge patch.

The NOAEL of approximately 135 mg/kg bw/day identified in the 2-year oral rat study provides the most health precautionary PoD from which to derive a TI for Irganox 1010.

TI = NOAEL/MF

MF = UF1 x UF2 x UF3

UF1 = 10 for inter-individual variation among humans.

UF2 = 10 for extrapolation from data derived in a species other than humans. [The default factor to extrapolate from data derived in rats is 5 according to ICH (2018).]

UF 3 = 50. A NOAEL from a good-quality chronic oral toxicity study with Irganox 1010 is used as the key PoD. No factor to account for study duration is required. According to ICH guidance, in the absence of data for the inhalation route of administration, MFs based on oral bioavailability may be used to derive tolerable intake levels. Irganox 1010 is poorly absorbed following oral administration to rats. According to one limited study, 2-3% of an ingested dose was absorbed in the gastrointestinal tract. As such, a conservative factor of 50 was used to account for the cross-route-extrapolation. There is a high degree of confidence on the current dataset for Irganox 1010, and there are several supporting studies.

TI = NOAEL/MF = 135 mg/kg bw/day/(10 x 10 x 50) = 0.027 mg/kg bw/day.

Consumer population	TI for Irganox 1010 (µg/kg bw/day)	Maximum exposure to Irganox 1010 (µg/kg bw/day)	Margin of Safety
Adults	27	0.14	193

As shown in the table above, the maximum exposure to Irganox 1010 resulting from vaping 30 mL e-liquid per day is approximately 200-fold lower than the TI. It can therefore be confidently concluded that exposure to Irganox 1010 is highly unlikely to pose any significant health risk to consumers.

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IRGANOX 1076

The GC-FID/MS and LC-DAD/MS analyses detected Irganox 1076 (CAS RN 2082-79-3) in the inner closure component (natural) at 7.08 µg/g. Consumers vaping 30 mL e-liquid per day may therefore be exposed at up to 23.56 µg/day, equivalent to approximately 0.39 µg/kg bw/day for a 60-kg individual.

Genotoxicity

Irganox 1076 did not cause chromosome aberrations in the bone marrow cells of hamsters (four/sex/group) given up to 2000 mg/kg bw/day for 2 days by gavage. No evidence of a dominant lethal mutation effect was found when male mice (20/group) were treated with up to 3000 mg/kg bw on a single occasion by gavage and then mated with untreated females each week over 6 consecutive weeks. An Ames test did not reveal any potential to induce mutations in four strains of *S. typhimurium*, with or without S9. No evidence of carcinogenicity was seen in lifetime studies when dietary Irganox 1076 was given at up to about 250 and 56 mg/kg bw/day to rats and mice (50/sex/group/species), respectively (3M Belgium BVBA/SPRL *et al*., 2019; OECD, 2006).

These data indicate that it is appropriate to assess Irganox 1076 as lacking mutagenic potential, a conclusion supported by the fact that it has been allocated a TDI by the SCF (EC, 2005).

Sensitisation

Experts from the Organisation for Economic Co-operation and Development concluded that Irganox 1076 was not a skin sensitiser in guinea pigs in a Maurer test, and that a human patch test confirmed this finding (OECD, 2006).

In the Maurer optimisation study, 20 guinea pigs were induced intradermally, three times weekly for 3 weeks, with a 0.1% solution of Irganox 1076 in a PEG/saline vehicle in the absence (first week) and presence (second and third week) of adjuvant. After a 14-day rest period, animals were challenged by injection with 0.1% Irganox 1076. Positive reactions were seen in 1/20 controls and 4/20 test animals, leading the experts to conclude that Irganox 1076 possesses no skin sensitising potential in guinea pigs (OECD, 2006). [A more accurate statement would be that the substance showed some evidence of a weak sensitising ability, in one test system, that was insufficient to warrant classification.]

The HRIPT, on 50 volunteers (26 males and 24 females), found no allergic skin reactions in response to the undiluted (but moistened) substance (OECD, 2006). A recent case of contact dermatitis has been linked to Irganox 1076 in a 10-year-old boy following surgery (as

detected by patch-testing with the substance at 0.05-2% in petrolatum) (Hattori *et al*., 2018); however, such cases appear to be extremely rare.

ADME considerations

OECD experts considered the oral bioavailability of Irganox 1076 to be of the order of 23-35% in the rat (OECD, 2006).

Repeated dose threshold toxicity

In a repeated dose inhalation study, rats (ten/sex/group) were exposed to an aerosol of Irganox 1076 at 0, 23, 128 or 543 mg/m^3 for 6 hours/day, 5 days/week for 3 weeks. There were no effects on appearance, behaviour, growth, survival, blood picture, serum chemistry, organ weights or the gross and microscopic appearance of a range of major tissues and organs, hence the NOAEC was 543 mg/m^3 (3M Belgium BVBA/SPRL *et al*., 2019; OECD, 2006). Given the rats weighed approximately 175 g, and assuming rats inhale 0.29 m^3 air/day (in line with ICH default assumptions), this NOAEC equates to an averaged systemic dose of 160 mg/kg bw/day[38].

In their deliberations over its use in food-packaging applications, SCF experts noted the existence of several subacute and subchronic oral rat studies (from 3 weeks to 3 months in duration), 2-year oral studies in mice and rats, and 2-generation and teratogenicity studies. From these, Irganox 1076 was allocated a TDI figure of 0.1 mg/kg bw [though details of the derivation were not provided] (EC, 2005).

Based on NOAELs of 30, 32-35 and 64-81 mg/kg bw/day in a 28-day rat study, a 90-day dog study and a 2-year rat study, respectively, OECD experts concluded that the overall NOAEL for oral exposure could be considered to be 30 mg/kg bw/day (OECD, 2006). In a 2-generation study in rats fed dietary Irganox 1076 over 10 months, there were no effects on the parents at about 100 mg/kg bw/day, on reproductive parameters at 315 mg/kg bw/day, or on pup growth and survival at about 30-40 mg/kg bw/day. Developmental studies in rats and mice treated by gavage on days 6-15 of pregnancy were reassuring, leading the OECD experts to propose 1000 mg/kg bw/day as the NOAEL for these effects in both species (OECD, 2006).

Health risk assessment

One inhalation toxicity study was identified in which rats were exposed to Irganox 1076 for 3 weeks. No adverse effects were identified at any tested concentration, hence the study NOAEC was the highest tested concentration of 543 mg/m^3, equivalent to an averaged systemic dose of approximately 160 mg/kg bw/day. This is therefore an appropriate PoD for the derivation of a TI for Irganox 1076.

TI = NOAEL/MF

MF = UF1 x UF2 x UF3

UF1 = 10 for inter-individual variation among humans.

[38] (543 mg/m^3 x 0.29 m^3/day x 6/24 x 5/7) / (0.175 kg) = 160 mg/kg bw/day.

UF2 = 10 for extrapolation from data derived in a species other than humans. [The default factor to extrapolate from data derived in rats is 5 according to ICH (2018).]

UF 3 = 10. A NOAEL from a good-quality subacute inhalation toxicity study with Irganox 1076 is used as the key PoD. According to ICH guidelines, for studies of less than 3 months, a factor of 10 to account for the short exposure duration is required. No cross-route extrapolation is required.

TI = NOAEL/MF = 160 mg/kg bw/day/(10 x 10 x 10) = 0.16 mg/kg bw/day.

Consumer population	TI for Irganox 1076 (µg/kg bw/day)	Maximum exposure to Irganox 1076 (µg/kg bw/day)	Margin of Safety
Adults	160	0.39	410

Although the above TI was derived by considering the only available PoD derived from inhalation data, ISO 10993-17 guidance indicates that for long-term exposures, it is appropriate to use chronic data, where available. Furthermore, lower NOAEL values were established in studies involving the oral route of exposure. As such, a second TI is derived below for Irganox 1076, as a health-precautionary measure.

The NOAEL of 64 mg/kg bw/day identified in the chronic oral toxicity investigation in rats appears to be another appropriate PoD.

TI = NOAEL/MF

Where MF = UF1 x UF2 x UF3

UF1 = 10 for inter-individual variation among humans.

UF2 = 10 for extrapolation from data derived in a species other than humans. [The default factor to extrapolate from data derived in rats is 5 according to ICH (2018).]

UF3 = 4. A NOAEL from a good quality chronic oral toxicity study is used as the key PoD. No factor to account for study duration is required. According to ICH guidance, MFs based on oral bioavailability may be used to derive tolerable intake levels. The oral bioavailability of Irganox 1076 was considered to be around 23-35% in rats. Assuming 100% absorption via the inhalation route, a factor of 4 is therefore an appropriate cross-route extrapolation factor.

TI = NOAEL/MF = 64 mg/kg bw/day/(10 x 10 x 4) = 0.16 mg/kg bw/day.

Consumer population	TI for Irganox 1076 (µg/kg bw/day)	Maximum exposure to Irganox 1076 (µg/kg bw/day)	Margin of Safety
Adults	160	0.39	410

As shown above, the two TIs generated for Irganox 1076 using different points-of-departure are identical. The maximum exposure to Irganox 1076 resulting from vaping 30 mL e-liquid per day is 410-fold lower than the TIs. Based on these highly reassuring margins of safety, it

can be confidently concluded that exposure to this extractable is highly unlikely to pose any significant health risk to consumers.

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N-PENTADECYLCYCLOHEXANE

The GC-FID/MS analysis detected n-pentadecylcyclohexane (CAS RN 6006-95-7) in the LDPE nozzle (natural) at 13.86 µg/g. Consumers vaping 30 mL e-liquid per day may therefore be exposed at up to 20.38 µg/day, equivalent to approximately 0.34 µg/kg bw/day for a 60-kg individual.

Despite comprehensive searches, no toxicity information was identified on this extractable.

As the n-alkyl group is highly unlikely to increase the toxicity potential of this compound, cyclohexane (CAS RN 110-82-7) is an appropriate read-across analogue. As such, the toxicology of cyclohexane is discussed below.

Genotoxicity

The ICH guideline for residual solvents places cyclohexane in class 2 ('solvents to be limited'), based on its inherent toxicological properties, and describes such substances as "non-genotoxic animal carcinogens" (ICH, 2018). According to OECD experts, cyclohexane is

not genotoxic *in vitro*, based on reassuring results in a series of studies including Ames bacterial tests, mouse lymphoma assays, a sister chromatid exchange assay, an unscheduled DNA synthesis assay and DNA damage in *E. coli*; an *in vivo* rodent bone marrow cytogenetic assay was also negative (OECD, 2000).

Moreover, Toxtree analysis did not identify any structural alerts for bacterial mutagenicity (Ames), genotoxic (or non-genotoxic) carcinogenicity, or micronuclei induction in rodents *in vivo* for n-pentadecylcyclohexane.

Overall, it is considered appropriate to assess this extractable as a threshold toxin.

Sensitisation

Based on the available data, OECD experts concluded that a very low sensitising potential can be anticipated for cyclohexane (OECD, 2000).

Moreover, Toxtree analysis did not identify any structural alerts (reactivity domains) for skin sensitisation for n-pentadecylcyclohexane.

ADME considerations

Cyclohexane is readily absorbed via inhalation and oral route, rapidly eliminated and not accumulated in the tissues. Metabolism occurs in the liver. Pulmonary elimination is the major route of excretion and a urinary excretion is also possible (OECD, 2000).

Repeated dose threshold toxicity

ICH's guideline for residual solvents recommends a PDE of 38.8 mg/day for cyclohexane (equal to 0.78 mg/kg bw/day for a 50-kg individual), and this figure applies to all routes of administration (ICH, 2018). An earlier publication indicates that the cyclohexane PDE was calculated from a NOEL of 97.2 mg/kg bw/day identified in a 10-week inhalation study (6 hours/day, 5 days/week) in rabbits. ICH applied UFs of 2.5 (for interspecies extrapolation; rabbit-to-human), 10 (for inter-individual variations) and 5 (for study duration). UFs of 1 and 1 were applied for effect severity and use of a NOEL, resulting in a PDE of 38.8 mg/day. (Connelly *et al.*, 1997).

Health risk assessment

The ICH PDE for cyclohexane (38.8 mg/day) is applicable to all exposure routes and is considered an appropriate basis for the current assessment. As such, the critical NOEL of 97.2 mg/kg bw/day, taken from the key 10-week inhalation study in rabbits (as selected by ICH experts), is taken as an appropriate PoD from which to derive the TI for n-pentadecylcyclohexane.

TI = NOAEL/MF

MF = UF1 x UF2 x UF3

UF1 = 10 for inter-individual variation among humans.

UF2 = 10 for extrapolation from data derived in a species other than humans. [The default factor to extrapolate from data derived in rabbits is 2.5 according to ICH (2018).]

UF 3 = 10. A NOEL from a 10-week inhalation toxicity study with cyclohexane is used as the key PoD. The study can be assumed to be of high quality, as this was identified as the key study by ICH experts. No cross-route-extrapolation is required. A factor of 5 is applied to account for the study duration. As a conservative measure, an additional factor of 2 is applied for the use of "read-across".

TI = NOAEL/MF = 97.2 mg/kg bw/day/(10 x 10 x 10) = 0.097 mg/kg bw/day.

Consumer population	TI for n-pentadecylcyclohexane (µg/kg bw/day)	Maximum exposure to n-pentadecylcyclohexane (µg/kg bw/day)	Margin of Safety
Adults	97	0.34	285

As shown in the table above, the maximum exposure to n-pentadecylcyclohexane resulting from vaping 30 mL e-liquid per day is 285-fold lower than the TI. It can therefore be confidently concluded that exposure to this extractable is highly unlikely to pose any significant health risk to consumers.

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PHTHALIC ACID CYCLIC OLIGOMER

The LC-DAD/MS analyses detected a 'phthalic acid cyclic oligomer', confirmed to be a cyclic molecule consisting of three ortho-phthalic acid units covalently linked (ester bonds) to three ethylene glycol units, in the PET containers (natural, solid black and translucent black) at a maximum of 24.4 µg/g. Consumers vaping 30 mL e-liquid per day may therefore be exposed at up to 217.4 µg/day, equivalent to approximately 3.6 µg/kg bw/day for a 60-kg individual.

Although no toxicity data were found on this oligomer itself, any absorbed cyclic ester is expected to hydrolyse, under physiological conditions, to phthalic acid (CAS RN 88-99-3) and ethylene glycol (CAS RN 107-21-1). Therefore, the toxicological profiles of both phthalic acid and ethylene glycol are considered in this assessment.

PHTHALIC ACID

Given that consumers will be exposed to the cyclic oligomer (MW 576 g/mol) at approximately 3.6 µg/kg bw/day, complete hydrolysis would yield phthalic acid (MW 166 g/mol) at 3.1 µg/kg bw/day.

Genotoxicity

Phthalic acid was not mutagenic in various Ames tests on *S. typhimurium* (including strains TA97, TA98, TA100, TA102, TA104, TA1535 and TA1537), with or without S9 (Argarwal *et al*., 1985; Lee and Lee, 2007; Sayato *et al*., 1987; Zeiger *et al*., 1992). It was also not genotoxic in chromosomal aberration tests in Chinese hamster ovary (CHO) cells (Lee and Lee, 2007; Phillips *et al*., 1982).

There was no significant induction of micronuclei in the erythrocytes of male mice given up to about 2100 mg/kg bw by intraperitoneal injection (Lee and Lee, 2007). Although phthalic acid produced dominant lethal mutations in male mice (Jha *et al*., 1998), the relevant study showed "methodological inadequacies and cannot be used for the assessment of germ cell mutagenicity", according to the German Research Foundation (DFG, 2012). The REACH dossier submitters concluded similarly (Chemical Inspection & Regulation Service Limited *et al*., 2019). While not mentioning the Jha *et al*. (1998) study specifically in their evaluation of the related chemical phthalic anhydride, experts from the OECD considered phthalic acid to be "non-genotoxic" (OECD, 2005).

No evidence of carcinogenicity was seen in rodents administered phthalic anhydride[39] at up to 1000 mg/kg bw/day (rats) or 4760 mg/kg bw/day (mice) via the diet (50/sex/species/group) for 105 weeks (NCI, 1979).

On that basis, phthalic acid is considered to be non-mutagenic.

Sensitisation

o-Phthalic acid showed no evidence of skin sensitising ability in a modified Maguire method. The test procedure consisted of topical application of 0.1 mL test material to the clipped and depilated skin of 10 Hartley guinea pigs on 4 days during a 10-day period. At the time of the third application, 0.2 mL of Freund´s adjuvant was injected intradermally adjacent to the insult site. After a 2-week rest period, the guinea pigs were challenged by dermal application of the test material on one flank and a solvent on the other flank. Examination of the challenge sites at 24 and 48 hours revealed no erythema or oedema and the test substance was concluded to be non-sensitising (Chemical Inspection & Regulation Service Limited *et al*., 2019).

Based on the available laboratory animal data, phthalic acid appears to lack significant sensitising ability. On that basis, the extractable is highly unlikely to cause sensitisation reactions in consumers.

[39] Phthalic anhydride is rapidly converted to phthalic acid in aqueous media (EFSA, 2018).

ADME considerations

According to German experts, only 20-30% of a single intragastric dose of o-phthalic acid was absorbed by rats, whereas p-phthalic acid is more extensively bioavailable via the oral route. Phthalic acid is mostly unchanged when excreted in the urine (DFG, 2012).

Repeated dose threshold toxicity

No inhalation toxicity studies were identified with o-phthalic acid, however, 28-day inhalation studies have been conducted with the meta- and para-isomers. Different effects[40] were observed in these studies, therefore it was considered appropriate (by the German expert reviewers) to treat the ortho-, meta- and para-isomers of phthalic acid separately (DFG, 2012).

No high-quality toxicity studies with o-phthalic acid were identified, however phthalic anhydride is rapidly converted to o-phthalic acid in aqueous media (EFSA, 2018; OECD, 2005). Two lifetime studies on phthalic anhydride are available. Apart from a <10% reduction in the growth of high-dose males, there were no treatment-related differences between test and control rats (50/sex/group) in a study providing dietary phthalic anhydride at doses of 0, 500 or 1000 mg/kg bw/day. The test included a microscopic examination of the organs and tissues. The health-precautionary NOAEL of 500 mg/kg bw/day (as given by OECD, 2005) is equivalent to about 560 mg/kg bw/day as phthalic acid (NCI, 1979; OECD, 2005).

In mice (50/sex/group) given time-weighted average (TWA) doses[41] of 2340 or 4670 mg/kg bw/day (males) or 1717 or 3430 mg/kg bw/day (females) of phthalic anhydride in the diet, a NOAEL could not be established due to various pathological effects that included lung and kidney lymphocytosis (both sexes), dose-related adrenal atrophy (males only) and mineralisation of the thalamus (males only). The study LOAEL was therefore the lowest tested TWA dose of 1717 mg/kg bw/day in females (NCI, 1979; OECD, 2005) [equivalent to about 1925 mg/kg bw/day as phthalic acid].

While there are no standard reproductive toxicity studies available for phthalic acid, it was noted by OECD experts that effects on the reproductive organs were not seen in the chronic rodent studies on phthalic anhydride[42] (NCI, 1979; OECD, 2005). Phthalic acid itself has been investigated in a prenatal test in which pregnant rats (eleven/group) were given diets containing about 0, 1000, 1700 or 3000 mg/kg bw/day on gestation days (GDs) 7-16, and were killed on GD20. Maternal toxicity exhibited as reduced growth in the mid-and high-dose group. Foetal weights were reduced in males and there were skeletal variations[43] in the high-dose group (where significant maternal toxicity was also observed) (Ema *et al*., 1997). The maternal NOAEL was 1000 mg/kg bw/day and the developmental NOAEL was 1700 mg/kg

[40] A concentration-dependent (minimal) degeneration of the tracheal epithelium was observed in rats exposed to p-phthalic acid from 0.52 mg/m^3, but no such effects were observed in an analogous study in which rats were exposed to m-phthalic acid at up to 10 mg/m^3 (DFG, 2012).

[41] Dietary concentrations were reduced at 32 weeks due to significant body weight effects. In females, 3570 mg/kg bw/day was reduced to 890 mg/kg bw/day (TWA 1717 mg/kg bw/day) and 7140 mg/kg bw/day was reduced to 1780 mg/kg bw/day (TWA 3430 mg/kg bw/day). In males, 3570 mg/kg bw/day was reduced to 1785 mg/kg bw/day (TWA 2340 mg/kg bw/day) and 7140 mg/kg bw/day was reduced to 3570 mg/kg bw/day (TWA 4670 mg/kg bw/day).

[42] There was a gross and microscopic examination of the preputial gland, prostate, seminal vesicle, testis and epididymis, and the mammary gland in male rats, and the mammary gland, uterus, endothelial gland, and ovary in female rats. In male mice, the epididymis was examined and in female mice the uterus and ovary.

[43] Slightly reduced vertebral ossification.

bw/day, and this was the basis for OECD experts considering phthalic anhydride "not a developmental toxicant" (OECD, 2005).

As mentioned in the genotoxicity section, intraperitoneal PA has produced dominant lethal mutations in male mice given up to 80 mg/kg bw/day for 5 days by intraperitoneal injection (dominant lethal test), and also sperm head abnormalities in mice given 300 mg/kg bw once (Jha *et al*., 1998). However, the relevant study showed "methodological inadequacies" and was not mentioned in respect of reproductive/developmental toxicity by the German Research Foundation (DFG, 2012).

Health risk assessment

As no relevant inhalation toxicity studies were identified, it is appropriate to consider the chronic oral toxicity of phthalic anhydride.

Although a NOAEL of 500 mg/kg bw/day was identified in the rat, the most health-precautionary TI would be derived by taking the LOAEL of 1925 mg/kg bw/day identified in mice as the critical PoD.

ICH (2018) guidelines indicate that a default factor of 10 should be applied to convert a LOAEL to the corresponding NOAEL value. As such the NOAEL-equivalent used in this assessment is 192.5 mg/kg bw/day.

TI = NOAEL/MF

MF = UF1 x UF2 x UF3

UF1 = 10 for inter-individual variation among humans.

UF2 = 12 for extrapolation from data derived in mice, in line with ICH PDE guidance. [The default factor to extrapolate from data derived in a species other than humans is 10 according to ISO 10993-17.]

UF 3 = 5. A LOAEL from a good-quality chronic oral toxicity study in mice with phthalic anhydride is used as the key PoD. This LOAEL has already been converted to an appropriate NOAEL-equivalent (see above). Phthalic anhydride rapidly hydrolyses to phthalic acid in contact with moisture, therefore this PoD is highly relevant and no UF to account for the use of a read-across analogue is required. No adjustment for study duration is required. A rat study indicated that o-phthalic acid is about 20-30% bioavailable via the oral route. As such, a factor of 5 to account for cross-route extrapolation is necessary.

TI = NOAEL/MF = 192.5 mg/kg bw/day/(10 x 12 x 5) = 0.32 mg/kg bw/day.

Consumer population	TI for phthalic acid (µg/kg bw/day)	Maximum exposure to phthalic acid (µg/kg bw/day)	Margin of Safety
Adults	320	3.1	103

As shown in the table above, the (theoretical) exposure to phthalic acid resulting from vaping 30 mL e-liquid per day is about 100-fold lower than the TI. It can therefore be confidently

concluded that exposure to phthalic acid from the phthalic acid cyclic oligomer is highly unlikely to pose any significant health risk to consumers.

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ETHYLENE GLYCOL

Given that consumers will be exposed to the cyclic oligomer (MW 576 g/mol) at approximately 3.6 µg/kg bw/day, complete hydrolysis would yield ethylene glycol (MW 62 g/mol) at 1.16 µg/kg bw/day.

Genotoxicity

Ethylene glycol was considered to be non-genotoxic by several Expert Groups, based on the results of several standard *in vitro* studies (including bacterial Ames tests for mutation, as well as chromosome aberration and gene mutation assays in mammalian cells) and *in vivo* studies (including chromosome aberration studies in rats and mice, a micronucleus assay in mice and a dominant lethal mutation assay in rats) (ATSDR, 2010; Danish EPA, 2013; IPCS, 2002; NICNAS, 2014; OECD, 2004; PHE, 2015).

Sensitisation

Ethylene glycol is not considered to have sensitising potential in humans, although some case reports have been identified (Danish EPA, 2013). Skin sensitisation was not induced in a guinea pig maximization test (GPMT) conducted according to OECD Test Guideline 406 (NICNAS, 2014).

ADME considerations

According to OECD experts the absorption estimate for inhaled ethylene glycol is approximately 100%. Furthermore, “ethylene glycols” are extensively absorbed by laboratory animals following ingestion, and the main metabolic pathway for metabolism of these compounds is oxidation via alcohol dehydrogenases and aldehyde dehydrogenases (ADH/ALD), resulting in oxalic and glycolic acids as well as carbon dioxide (OECD, 2004).

Repeated dose threshold toxicity

The ICH guideline for residual solvents recommends an acceptable level of ethylene glycol in pharmaceutical products that is considered safe for patients. It was placed in class 2 for 'solvents to be limited' based on its inherent toxicological properties. A PDE of 3.1 mg/day was derived and this figure applies to all routes of administration (ICH, 2018). It appears that this figure was derived from a study where malformations and reduced foetal body weights were observed at all oral dose levels of a prenatal developmental toxicity study in mice. MFs of 12 (interspecies differences; mouse-to-human), 10 (inter-individual differences), 10 (severity of effect; teratogenicity) and 10 (quality of data) were applied to the LOAEL of 750 mg/kg bw/day to yield a figure of 0.0625 mg/kg bw/day, which equated to a PDE of 3.1 mg/day for a 50-kg individual (Connelly *et al.*, 1997).

Long-term oral health-based guidance-values (HBGVs) have been recommended by several authoritative groups, each based on nephrotoxicity observed in laboratory animals. These include a TI of 0.05 mg/kg bw/day (Health Canada, 2000), TDI values of 0.05 mg/kg bw/day (IPCS, 2002) and 0.5 mg/kg bw/day (SCF, 1986, 2002), and an earlier chronic reference dose (RfD[44]) of 2 mg/kg bw/day (US EPA, 1987). In addition, scientists from the US Agency for Toxic Substances and Disease Registry (ATSDR) considered that its acute-duration minimal risk level (MRL) (0.8 mg/kg bw/day) was protective for chronic kidney effects (ATSDR, 2010).

Scientists for the International Programme on Chemical Safety (IPCS, 2002) adopted Health Canada's (2000) benchmark dose approach to derive a BMD_{05} (i.e. the dose estimated to cause a 5% increase in incidence of histopathological changes in the kidneys of male rats) of 49 mg/kg bw/day. Three UFs, each of 10, were then applied to account for intra- and inter-species variation as well as extrapolation to a chronic exposure, as rats were treated for 16 weeks in the critical study, giving a tolerable exposure level for man of 0.05 mg/kg bw/day.

Health risk assessment

Several Expert Groups have identified kidney effects in male rats to be the most sensitive toxicity induced by ethylene glycol. The most-health precautionary HBGVs were recommended by Health Canada (2000) and IPCS (2002), who initially derived a BMD_{05} value of 49 mg/kg bw/day (effectively equivalent to a NOAEL), based on histopathological effects in the kidneys of male rats identified in a 16-week oral toxicity study. This value is more conservative than the LOAEL of 750 mg/kg bw/day used by the ICH to derive its PDE, and is therefore selected as the critical POD for calculation of the TI.

TI = NOAEL/MF.

MF = UF1 x UF2 x UF3.

UF1 = 10 for inter-individual variation among humans.

[44] In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

UF2 = 10 for extrapolation from data derived in a species other than humans. [The default factor to extrapolate from data derived in rats is 5 according to ICH (2018).]

UF3 = 5. The data are of good quality. A BMD_{05} (essentially equivalent to a NOAEL) from a subchronic oral toxicity study is used as the key POD. Ethylene glycol is readily absorbed following ingestion and distributed throughout the body, so further adjustment to reflect absorption difference between routes is not required. There are many supporting studies with higher NOAELs reported, and several expert groups agree that nephropathy is the critical toxicological endpoint for ethylene glycol. A factor of 5 for study duration is applied (in line with the ICH (2018) default) for extrapolating from a subchronic rodent study NOAEL to lifetime exposure.

TI = NOAEL/MF = 49 mg/kg bw/day/(10 x 10 x 5) = 0.098 mg/kg bw/day.

Consumer population	TI for ethylene glycol (µg/kg bw/day)	Maximum exposure to ethylene glycol (µg/kg bw/day)	Margin of Safety
Adults	98	1.16	84

As shown in the table above, the (theoretical) exposure to ethylene glycol resulting from vaping 30 mL e-liquid per day is 84-fold lower than the TI. It can therefore be confidently concluded that exposure to ethylene glycol from the phthalic acid cyclic oligomer is highly unlikely to pose any significant health risk to consumers.

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C8-C20 ACYCLIC ALKANE CATEGORY

The GC-FID/MS analyses detected 2-methyloctadecane (CAS RN 1560-88-9), 5-ethyl-2-methyloctane (CAS RN 62016-18-6) and pentadecane (CAS RN 629-62-9) in the black inner closure component; decane (CAS RN 124-18-5), dodecane (CAS RN 112-40-3), eicosane (CAS RN 112-95-8), hexadecane (CAS RN 544-76-3), octadecane (CAS RN 593-45-3) and tetradecane (CAS RN 629-59-4) in the natural inner closure component; tridecane (CAS RN 629-50-5) in the natural LDPE nozzle component; undecane (CAS RN 1120-21-4) in the natural PP nozzle component; and dodecane, tridecane and undecane in the translucent black LDPE nozzle component. Their structural similarity (branched/linear alkanes, with chain length C8-C20) and likely common toxicological mode of action (MoA) indicates that it is health precautionary to assess them together.

Consumers vaping 30 mL e-liquid per may therefore be exposed to these alkane extractables at a maximum combined exposure of 481 µg/day[45], equivalent to approximately 8.0 µg/kg bw/day for a 60-kg individual.

Genotoxicity

A lack of genotoxic potential for these substances is firmly supported by numerous genotoxicity screening studies. For example, OECD assessments on various alkane groupings, including "C7-C9 Aliphatic Hydrocarbon Solvents Category" (OECD, 2010), "C9-C14 Aliphatic [≤2% aromatic] Hydrocarbon Solvents Category" (OECD, 2012), and "C14-C20 Aliphatic [≤2% aromatic] Hydrocarbon Solvents Category" (OECD, 2011), overwhelmingly considered these substances to be of low genotoxicity concern. EFSA also had no concerns over genotoxicity in its assessment of several alkanes, including dodecane and tetradecane, as part of the EU food flavourings evaluation programme (EFSA, 2015).

Moreover, REACH dossiers on "C4-C10 branched and linear hydrocarbons (light) – Naphtha" (CAS RN 848301-65-5; CHOREN Fuel Freiberg GmbH & Co. KG, 2018a), "C8-C16 branched and linear hydrocarbons (full range) – Kerosine" (CAS RN 848301-66-6; Chemservice GmbH (9APM), 2018; CHOREN Fuel Freiberg GmbH & Co. KG, 2018b), and "C8-C26 branched and linear hydrocarbons – Distillates" (CAS RN 848301-67-7; Chemservice GmbH (9APM) *et al*., 2019) summarised a small number of studies where the test materials did not induce bacterial mutations (in five strains of *S. typhimurium* or an *E. coli* strain), micronuclei or chromosome aberrations in human lymphocytes in culture, or chromosome aberrations in the bone marrow of rats treated *in vivo*. The substances were used in a "read-across" manner between dossiers. The REACH dossiers on decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, octadecane and eicosane summarise a wide range of *in vivo* and *in vitro* tests, in which no evidence of genotoxicity was observed (BIOSYNTHIS PRODUCTION *et al*., 2019; BP Europa SE *et al*., 2019; CEPSA Química, S.A. *et al*., 2019a, 2019b; DHW Deutsche Hydrierwerke GmbH *et al.*, 2019; Haltermann Carless Deutschland GmbH *et al*., 2019; Merck Schuchardt OHG *et al.*, 2019; Sasol Germany GmbH, 2019; Sasol Italy S.p.A., 2019).

Overall, it is clearly appropriate to assess these aliphatic hydrocarbons as threshold toxins.

Sensitisation

In the absence of sensitisation data on any of the ten detected alkanes, a Toxtree analysis (version 3.1.0, with plug-ins) was performed. No structural alerts for skin sensitisation were present for any of these substances. Relevant REACH dossiers summarise a variety of sensitisation studies on similar materials[46]. The key studies in laboratory animals appear to be three separate guideline maximization tests (one on 20, two each on 30 guinea pigs), in which no evidence of sensitisation was observed (BIOSYNTHIS PRODUCTION *et al*., 2019; BP Europa SE *et al*., 2019; CEPSA Química, S.A. *et al*., 2019a, 2019b; DHW Deutsche Hydrierwerke GmbH *et al.*, 2019; Haltermann Carless Deutschland GmbH *et al*., 2019; Merck Schuchardt OHG *et al.*, 2019; Sasol Italy S.p.A., 2019).

[45] Maximum exposure to C8-C20 acyclic alkanes results from consistent use of (10 mL) CCS with natural inner closure and LDPE (translucent black) nozzle components.

[46] Shellsol TD, MRD-83-205 and MRD-83-206. These substances are described in the REACH dossier as "C9-C14 aliphatic, <2% aromatic hydrocarbons".

In a category assessment of "C14-C20 Aliphatic [<2% aromatic] hydrocarbon solvents", OECD experts noted the availability of seven studies of several hydrocarbon solvents in human volunteers. Clinical tests on groups of 24-112 patients found no sensitisation effects and the C14-C20 aliphatic hydrocarbons were not expected to be sensitisers (OECD, 2011). A similar conclusion was reached for the C7-C9 and C9-14 aliphatic hydrocarbon groups (OECD, 2010, 2012).

The detected alkanes are not considered to pose any significant sensitisation risk to consumers.

ADME considerations

Due to their lipophilicity and relatively low molecular weight, EFSA considered that aliphatic hydrocarbons may be assumed to be absorbed in the gastrointestinal tract and subsequently be metabolised to polar oxygenated substances (EFSA, 2015). OECD experts stated that C7-C9 alkanes are readily absorbed and distributed throughout the body (OECD, 2010), and estimated that 61-81% of a C9-C14 hydrocarbon solvent would be absorbed following ingestion (OECD, 2012).

Repeated dose threshold toxicity

Aliphatic hydrocarbons generally possess a low order of systemic toxicity. The toxicity potential tends to decrease with increasing chain length. The ICH guideline for residual solvents recommends acceptable levels for the short-chain alkanes pentane and heptane in pharmaceutical products. Both substances were placed in class 3 for 'solvents with low toxic potential'. These substances therefore inherit a default PDE of 50 mg [1 mg/kg bw for a 50-kg individual[47]] (or higher), applicable to any exposure route (ICH, 2018). Substance-specific PDEs were also derived in an earlier publication. For pentane, a PDE was calculated from a critical no-observed-effect level (NOEL) of 3023 mg/kg bw/day, identified in a 16-week (12 hours/day) inhalation study in rats. ICH applied MFs of 5 (for interspecies extrapolation; rat-to-human), 10 (for inter-individual variations) and 5 (for study duration). MFs of 1 and 1 were applied for effect severity and use of a NOEL, resulting in a PDE of 604.6 mg/day. The PDE for heptane was derived from an analogous inhalation study. A total MF of 250 was applied to the NOEL of 4196 mg/kg bw/day to yield a PDE of 840 mg/day (Connelly *et al.*, 1997).

Health risk assessment

In general, short-chain alkanes are of low toxicological concern. The "default" ICH PDE figure for pentane and heptane (equivalent to 1 mg/kg bw/day, by any exposure route) would therefore be considered representative and very health-precautionary for the majority of various aliphatic hydrocarbons under consideration. A group TI of 1 mg/kg bw/day is therefore applied here.

Consumer population	Group TI for alkanes category (µg/kg bw/day)	Maximum combined exposure to alkanes (µg/kg bw/day)	Margin of Safety
Adults	1000	8.0	125

[47] 50 kg is the default body weight used by ICH.

As shown in the table above, the maximum combined exposure to the group of alkanes resulting from vaping 30 mL e-liquid per day is 125-fold lower than the group TI. It can therefore be confidently concluded that exposure to 2-methyloctadecane, 5-ethyl-2-methyloctane, decane, dodecane, eicosane, hexadecane, octadecane, pentadecane, tetradecane, tridecane and undecane is highly unlikely to pose any significant health risk to consumers.

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FATTY AMIDES CATEGORY

The GC-FID/MS analysis detected 13-docosenamide (erucamide; CAS RN 112-84-5) in the natural inner closure component, and 9-octadecenamide (oleamide; CAS RN 301-02-0; 3322-62-1), hexadecanamide (palmitamide; CAS RN 629-54-9) and octadecanamide (stearamide; CAS RN 124-26-5) in the natural and/or translucent black LDPE nozzle components. Due to their close structural similarity and likely similar MoA, these long-chain fatty amides (saturated and unsaturated) have been grouped as a category, and assessed together at a combined exposure.

Consumers vaping 30 mL e-liquid per day may therefore be exposed at up to 238 µg/day[48], equivalent to approximately 3.96 µg/kg bw/day for a 60-kg individual.

Genotoxicity

Oleamide, erucamide and stearamide showed no evidence of mutagenicity in Ames tests on *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 and stearamide was also

[48] Maximum exposure to fatty amides results from consistent use of (10 mL) CCS with natural inner closure and natural LDPE nozzle components.

inactive in *E. coli* strain WP2 uvr A/pKM101. In good-quality *in vitro* studies[49], erucamide did not cause gene mutations in mouse lymphoma cells or chromosome aberrations in hamster lung cells. In each case, testing was carried out with and without S9 (Basell Sales & Marketing B.V. *et al.*, 2019; Croda Europe Limited *et al.*, 2019; ECCC/HC, 2019; JETOC, 2000; US EPA, 2004, 2010).

A mixture of methyl-branched and linear C14-C18 alkanamides[50] gave no evidence of genotoxicity in a micronucleus test. It was administered by intraperitoneal injection to groups of seven male mice at 0, 100, 200 or 400 mg/kg bw. Based on a reduction in the polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) ratio, some components of the mixture reached the bone marrow, but they did not produce any treatment-related chromosome damage in the PCE (EFSA, 2017).

Overall, it was considered appropriate to assess these fatty amides as non-mutagenic threshold toxins.

Sensitisation

In a murine local lymph node assay (LLNA), erucamide gave no evidence of skin sensitising potential when applied at up to 25% (in tetrahydrofuran) to the ears of female mice (five/concentration) for 3 consecutive days. The study was conducted in compliance with OECD Test Guideline 429[51] (Basell Sales & Marketing B.V. *et al.*, 2019).

A cook who experienced contact urticaria (hives) at work developed a wheal and flare reaction following prick- and scratch-tests with several glove extracts and erucamide. No reactions to the extracts (or to erucamide) were seen when the testing was extended to three healthy volunteers (Sugiura *et al.*, 2002). Another woman, who experienced skin eruptions related to jewellery and plastic, developed severe urticarial reactions when patch-tested with plastics that contained oleamide or BHT[52]. A "very strong urticarial reaction" was seen after skin application of 0.1% oleamide (in ethanol). However, 15 other patients did not react to patch tests with 1% oleamide (in ethanol). The investigator noted that, prior to this case, "neither delayed nor immediate hypersensitivity to [oleamide] has been reported". He also stated that the immediate reactions to three unrelated chemicals "may give rise to doubt about an immunological mechanism" (Osmundsen, 1980). No more recent published cases have been noted.

On this basis, sensitisation effects from the potential exposure to these structurally-related extractables are extremely unlikely.

ADME considerations

A "digestibility" study indicated that the gastrointestinal absorption of erucamide when administered at 10% in the diet of a group of five male rats for 4 weeks was of the order of

[49] Compliant with (earlier versions of) OECD Test Guidelines 476 (*In Vitro* Mammalian Cell Gene Mutation Test) and 473 (*In Vitro* Mammalian Chromosome Aberration Test) respectively.

[50] It was composed of isooctadecanamide (predominantly methyl branched) and stearamide, along with minor amounts of palmitamide, myristamide, polybranched isooctadecanamide, and the amides of arachidic and behenic acid.

[51] Skin Sensitisation. Local Lymph Node Assay.

[52] Butylated hydroxytoluene.

60%. The extent of absorption was estimated by weekly determinations of faecal fat content (Basell Sales & Marketing B.V. *et al.*, 2019).

Repeated dose threshold toxicity

The REACH registration dossier for erucamide summarises a rat study conducted in compliance with OECD Test Guideline 408[53] in which groups of ten males and ten females were gavaged with erucamide (in corn oil) at 0, 100, 300 or 1000 mg/kg bw/day for 90 days. The routine microscopic examination of the tissues from a comprehensive range of organs from the control and high-dose group revealed no evidence of toxicity (NOAEL 1000 mg/kg bw/day) (Basell Sales & Marketing B.V. *et al.*, 2019).

In a study conducted in compliance with OECD Test Guideline 414[54], erucamide had no adverse developmental effects when given at 0, 100, 300 or 1000 mg/kg bw/day, by gavage, to female rats (20-23/group) on GD 5-19. There was no indication of maternal toxicity at the maximum tested dose (Basell Sales & Marketing B.V. *et al.*, 2019).

Health risk assessment

The NOAEL of 1000 mg/kg bw/day identified in the 90-day oral rat study with erucamide appears to be an appropriate and representative PoD from which to derive a group TI for this category of fatty amides.

TI = NOAEL/MF

MF = UF1 x UF2 x UF3

UF1 = 10 for inter-individual variation among humans.

UF2 = 10 for extrapolation from data derived in a species other than humans. [The default factor to extrapolate from data derived in rats is 5 according to ICH (2018).]

UF3 = 20. The data are of good quality. A health-precautionary NOAEL from a good-quality subchronic oral toxicity study with erucamide is used as the key PoD. ICH (2018) guidelines recommend a factor of 5 is appropriate to account for differences in study duration when data are derived from subchronic rodent studies. This factor is therefore applied here. Erucamide is absorbed to the extent of about 60% following ingestion, approximately in-line with the ECHA (2012) recommended default for oral absorption of 50%. As such, a factor of 2 to reflect absorption difference between routes appears to be appropriate. As the key data were derived with erucamide, a closely related long-chain fatty amide, a health-precautionary factor of 2 is also applied to account for “read-across” uncertainties.

TI = NOAEL/MF = 1000 mg/kg bw/day/(10 x 10 x 20) = 0.5 mg/kg bw/day.

Consumer population	TI for fatty amides (µg/kg bw/day)	Maximum exposure to fatty amides category (µg/kg bw/day)	Margin of Safety
Adults	500	3.96	126

[53] Repeated Dose 90-Day Oral Toxicity Study in Rodents.

[54] Prenatal Developmental Toxicity Study.

As shown in the table above, the potential exposure to erucamide, oleamide, palmitamide, stearamide resulting from vaping 30 mL e-liquid per day is 126-fold lower than the highly conservative TI. It can therefore be confidently concluded that exposure to these fatty amide extractables is highly unlikely to pose any significant health risk to consumers.

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FATTY ACIDS CATEGORY

The GC-FID/MS analyses detected hexadecanoic acid (palmitic acid; CAS RN 57-10-3) and octadecanoic acid (stearic acid; CAS RN 57-11-4) in the inner closure (natural and black) and PP nozzle (natural and translucent black) (only used for the 30-mL device) components. Consumers vaping 30 mL e-liquid per may be exposed to these fatty acid extractables at a maximum combined exposure of 63.86 µg/day[55], equivalent to approximately 1.0 µg/kg bw/day for a 60-kg individual.

These substances are members of a structurally related group of saturated fatty acids, which are synthesised by mammals and plants and are normal components of both the body and the diet, occurring in many animal and vegetable fats and oils, usually as triglycerides. Palmitic acid is the most common fatty acid in animals and plants, while humans typically consume about 6-8 g/day of stearic acid (EFSA, 2017; OECD, 2014). Their structural similarity and likely common toxicological MoA indicates that it is health precautionary to assess them together.

Genotoxicity

In an assessment of a large group of aliphatic acids, including palmitic and stearic acids, members of this group of compounds were not anticipated to be genotoxic based on mutagenicity and clastogenicity data, both *in vitro* and *in vivo* (OECD, 2014). More recently, reviews by EFSA and the US Cosmetic Ingredient Review Expert Panel have concluded a lack of genotoxicity concern for groups of C8-C18 fatty acids and C8-C22 fatty acids (and some salts), respectively, including caprylic, capric, lauric, myristic, palmitic, stearic and oleic acid[56] (CIR, 2019; EFSA, 2017).

In bacterial reverse mutation (Ames) assays, octanoic (C8), decanoic (C10), dodecanoic (C12), tetradecanoic (C14), stearic (C18) and oleic acids (also C18) showed no ability to mutate various strains of *S. typhimurium* (including TA97, TA98, TA100, TA1535, TA1536, TA1537 and TA1538), when tested with and without S9 (EFSA, 2017). In addition, no evidence of mutagenicity was observed when *E. coli* (WP2uvrA) bacteria were treated with stearic and oleic acids in other good-quality Ames tests, again with and without S9 (EFSA, 2017; Shimuzu *et al.*, 1985).

As such, palmitic acid and stearic acid are not considered to concerns for genotoxic carcinogenicity, and they have therefore been assessed here as threshold toxins.

Sensitisation

The recent EFSA toxicity review on fatty acids (including palmitic and stearic acid) did not mention any concerns over sensitisation (EFSA, 2017). No structural alerts for skin sensitisation reactivity domains were identified, for either fatty acid, in a Toxtree analysis (version 3.1.0 with plug-ins). Given the large amount of these fatty acids, either free or as glycerides, in the body, diet and consumer products (e.g. cosmetics), without documented

[55] Maximum exposure to fatty acids results from consistent use of (30 mL) CCS with natural inner closure and translucent black PP nozzle components.

[56] Elevated levels of etheno-DNA adducts were seen in the colon cells (both sexes) and white blood cells (females only) after oleic acid was administered orally to rats at 500 mg/kg bw/day for 30 days; however, EFSA considered that oleic acid was not directly involved in the production of these DNA adducts (EFSA, 2017).

indication of any sensitisation reactions it would be very surprising if they were to possess any such potential.

The REACH dossier for stearic acid cites an unreferenced report of a US CIR Panel Working Group [presumably CIR (1987)] stating the following: "Results from topical application of Oleic, Palmitic, and Stearic Acid to the skin of mice, rabbits, and guinea pigs produced little or no apparent toxicity. Studies using product formulations containing Oleic and Stearic acids indicate that neither is a sensitizer or photosensitizing agent. Cosmetic product formulations containing Oleic, Laurie, Palmitic, and Stearic Acids at concentrations ranging up to 13% were not primary or cumulative irritants, nor sensitizers". The dossier also summarises a human maximization test[57] involving five 24-hour covered applications of a 5% formulation of an unspecified acid in petrolatum (induction phase) and a covered 48-hour challenge patch test with a 1% concentration; no evidence of sensitisation was seen (3M Belgium BVBA/SPRL *et al.*, 2019).

The REACH dossier for palmitic acid summarises a Buehler test on 20 guinea pigs and an unnamed acid, a maximization test on 20 guinea pigs and lauric (C12) acid, and a maximization test on 10 guinea pigs with azelaic acid. No sensitisation reactions were seen in the total of 50 animals. The human maximization study summarised in the stearic acid dossier also appears in this palmitic acid dossier (AAK Sweden AB *et al.*, 2019).

ADME considerations

Fatty acids are readily and extensively absorbed from the gastrointestinal tract, after which they either undergo metabolism or are incorporated into chylomicrons, which enter the systemic circulation. Ultimately, fatty acids, either incorporated into glycerides and phospholipids, are catabolised via the beta-oxidation pathway and the tricarboxylic acid cycle to carbon dioxide which is finally excreted via exhalation (EFSA, 2017).

Repeated dose threshold toxicity

The low oral and systemic toxicity of palmitic and stearic acid is reflected in the allocation of an "ADI [Acceptable Daily Intake] Not Limited"[58] status (in simple salt form e.g. the sodium, potassium, calcium etc., salts) by JECFA in 1973 (JECFA, 1974a, 1974b). Later, JECFA allocated an ADI "not specified" status to dodecanoate, tetradecanoate, palmitate and stearate moieties (JECFA, 1986). More recently, EFSA has concluded that there is no evidence of toxic effects of fatty acids in subchronic toxicity feeding studies at doses of up to 10% in the diet (EFSA, 2017).

This EFSA (2017) conclusion was based on several studies. In one, no adverse effects were observed when male rats (5/group) were fed dodecanoic (lauric) acid in their diet at 0 or 9000 mg/kg bw/day for 18 weeks. The animals were weighed, observed for overt signs of toxicity, analysed for haematological and gross pathological changes, and organs were

[57] A sensitisation protocol.

[58] The term "ADI not limited" is no longer used by JECFA, and has been replaced by "ADI not specified". "ADI not specified" is the most favourable classification, and is used for "a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of JECFA, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an acceptable daily intake expressed in numerical form is not deemed necessary."

weighed (Fitzhugh *et al.*, 1960). When albino rats (mixed strains; 10/sex/group) were given a diet containing decanoic, dodecanoic or palmitic acid at 9000 mg/kg bw/day for 150 days, no remarkable changes were detected in the forestomach or glandular stomach after interim sacrifices were performed throughout the study (Mori, 1953). A microscopic "foreign body-type reaction" in adipose tissue was reported after 8 weeks in rats exposed to a diet containing 50.4% palmitic or stearic acid ("very high doses") for 24 weeks (Herting and Crain, 1958; Herting *et al.*, 1959).

Industry's REACH dossiers on palmitic and stearic acids include long-term proposed derived no-effect levels[59] (DNELs) for systemic effects in the general population of 5 mg/kg bw/day, 2.5 mg/kg bw/day and 4.3 mg/m^3 for the dermal, oral and inhalation routes, respectively, in each case based on "repeated dose toxicity" [by the oral route]. All of these DNELs were derived from the same key, high-quality (OECD TG422[60]) oral study with docosanoic acid (C22) (CAS RN 112-85-6). No adverse effects were observed at any dose level when rats (13/sex/group) were administered this C22 fatty acid, daily, by oral gavage for 42 (males) or about[61] 43-50 (females) days at 0, 100, 300 or 1000 mg/kg bw/day. The NOAEL was identified as 1000 mg/kg bw/day, the highest dose level tested, and a LOAEL was not established (3M Belgium BVBA/SPRL *et al.*, 2019; AAK Sweden AB *et al.*, 2019).

Health risk assessment

In the absence of good-quality studies on either palmitic or stearic acid, and no inhalation data, the high-quality oral study on a slightly longer fatty acid (docosanoic acid) where a NOAEL of 1000 mg/kg bw/day was established in rats gavaged daily for at least 42 days, was selected as the key study. This NOAEL was taken as a suitable PoD.

TI = NOAEL/MF

MF = UF1 x UF2 x UF3

UF1 = 10 for inter-individual variation among humans.

UF2 = 10 for extrapolation from data derived in a species other than humans. [The default factor to extrapolate from data derived in rats is 5 according to ICH (2018).]

UF3 = 10. A NOAEL from a good-quality subchronic oral toxicity study with docosanoic acid is used as the key PoD. ICH (2018) guidelines recommend a factor of 10 to account for differences in study duration when data are derived from rodent studies of less than 3 months. This factor is therefore applied here. With regards to extrapolation from an oral route to an inhalation exposure, as fatty acids are extensively absorbed via the oral route, no cross-route extrapolation factor is applied. Many supporting studies with fatty acids are available in the literature.

TI = NOAEL/MF = 1000 mg/kg bw/day/(10 x 10 x 10) = 1 mg/kg bw/day.

[59] A DNEL is an exposure level that should not be exceeded. If exposure is kept below a DNEL, the health risks are considered to be adequately controlled.

[60] Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test. Current version adopted 29 July 2016.

[61] Not disclosed in the REACH dossier.

Consumer population	TI for fatty acids category (µg/kg bw/day)	Maximum exposure to fatty acids (µg/kg bw/day)	Margin of Safety
Adults	1000	1.0	1000

As shown in the table above, the maximum combined exposure to hexadecanoic acid and octadecanoic acid resulting from vaping 30 mL e-liquid per day is 1000-fold lower than the highly conservative TI. It can therefore be confidently concluded that exposure to these two structurally related substances is highly unlikely to pose any significant health risk to consumers.

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BARIUM

The ICP-MS analysis detected barium (CAS RN 7440-39-3) in the container (natural and translucent black), inner closure (natural), PP nozzle (natural) and LDPE nozzle (natural) components. Consumers vaping 30 mL e-liquid per day by filling up their ENDS using the 10 mL CCS (with the natural container, natural inner closure and natural LDPE nozzle) may therefore be exposed at up to 0.55 µg/day, equivalent to approximately 9 ng/kg bw/day for a 60-kg individual.

Barium is nutritionally non-essential, and no metabolic function is known.

The toxicity of barium compounds depends on their solubility. The free ion is readily absorbed and mainly accumulates in the skeleton. Acute or chronic exposure to barium salts results in a number of disorders, including renal intoxication, hypertension, cardiac malfunction, and hearing loss in experimental animals. The kidney appears to be the most sensitive toxicity target in rats and mice following repeated ingestion of soluble barium salts (Nordberg *et al.*, 2018). Canadian experts have recently proposed a maximum acceptable concentration (MAC) for barium of 2 mg/L in drinking water, based on kidney toxicity in mice (Health Canada, 2018). Assuming an individual consumes 2 L drinking water per day, this MAC would provide a systemic dose of 4 mg/day.

ICH utilised epidemiological data (based on drinking water levels) in deriving an oral PDE for barium. Cardiovascular and kidney effects were absent in populations whose drinking water contained a mean barium concentration of up to 7.3 mg/L, equivalent to 14.6 mg/day assuming a water consumption of 2 L/day. A MF of 10 was applied for sensitive subpopulations, yielding a rounded oral PDE of 1400 µg/day (ICH, 2019).

In the absence of any relevant inhalation data on barium salts, ICH utilised the US Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL) of 500 µg/m^3 when deriving its inhalation PDE for barium[62]. As the PEL is a time weighted average (TWA) value, it was extrapolated to a continuous concentration of 119 µg/m^3 and subsequently converted to a systemic dose of 68.8 µg/kg bw[63]. ICH then applied a UF of 10 to account for variability between individuals to achieve a PDE of 6.86 µg/kg bw/day (ICH, 2019).

The ICH (inhalation) PDE can be adopted as the TI for barium.

Consumer population	TI for barium (µg/kg bw/day)	Maximum exposure to barium (µg/kg bw/day)	Margin of Safety
Adults	6.86	0.009	762

As shown in the table above, the maximum exposure to barium resulting from vaping 30 mL e-liquid per day is 762-fold lower than the TI. It can therefore be confidently concluded that exposure to barium is highly unlikely to pose any significant health risk to consumers.

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CHROMIUM

The ICP-MS analysis detected chromium (with valency unspecified, CAS RN 7440-47-3) in the natural and translucent black containers at 7.4 and 25.57 ng/g, respectively, as well as the translucent black LDPE nozzle at 57.14 ng/g. Consumers vaping 30 mL e-liquid per day by filling up their ENDS using the 10 mL CCS (with translucent black container and translucent

[62] These PEL limits are published by the US Department of Labor.

[63] (500 µg/m^3 x 8/24 x 5/7 x 28.8 m^3/day) / 50 kg bw.

black LDPE nozzle) may therefore be exposed to up to 0.312 µg/day, equivalent to approximately 5.2 ng/kg bw/day for a 60-kg individual.

Chromium is found in a variety of oxidation states. Cr(III) is the most abundant environmental form, and plays a role in glucose metabolism in mammals. ICH experts have considered sources of chromium in pharmaceutical products, including as a leachable from equipment or container closure systems, and have noted that, unless it is used as a catalyst during the drug manufacturing process, chromium will be in the form of metallic chromium (Cr(0)) or Cr(III) rather than the more toxic Cr(VI) (ICH, 2019) [see below for further details].

Chromium has been extensively studied by Expert Groups and a number of tolerable exposure values are available for both the III and VI valency states. EFSA derived an oral TDI of 300 µg/kg bw for Cr(III) from reliable 2-year studies on rats and mice fed chromium picolinate (EFSA, 2014; NTP, 2010). The same data were used by ICH to calculate a parenteral PDE value of 1100 µg/day for chromium (excluding Cr(VI) compounds). An inhalation PDE of 3 µg/day (rounded from 2.9 µg/day; 0.058 µg/kg bw/day for a 50-kg individual) was derived for Cr(III) based on a subchronic inhalation study in rats with Cr(III) sulphate (ICH, 2019). Previously, the US EPA had derived a chronic oral RfD of 1500 µg/kg bw/day for insoluble Cr(III) salts, based on a chronic feeding study in rats given Cr(III) oxide [it is likely that this RfD will change when reassessed] (US EPA, 1998a). Experts from the EMA adopted a "conservative approach" in deriving an oral PDE of 250 µg/day for (all valencies of) chromium, utilising the TDI for Cr(VI) of 5 µg/kg bw/day as calculated by the Dutch RIVM (2001) and assuming a standard body weight of 50 kg. A parenteral PDE of 25 µg/day was estimated, based on an oral bioavailability of 10%. The Agency considered Cr(VI) to pose a genotoxic carcinogenicity hazard via inhalation exposure, and derived an inhalation PDE of 10 ng/day [see next paragraph]; it was noted that Cr(VI) was non-carcinogenic in limited long-term oral studies (EMA, 2008). This document has now been superseded.

In general, a greater degree of toxicity is expected from Cr(VI) than from Cr(III); Cr(VI) being a known human carcinogen following exposure by the inhalation route (IARC Group 1) (IARC, 2012). In a recent EC SCOEL assessment, it was concluded that "Cr(VI) compounds are carcinogens with no threshold" (SCOEL, 2017). Various Expert Groups consider Cr(VI) to induce tumours by a genotoxic mechanism and have set HCVs for inhalation exposure (DECOS, 2016; ECHA, 2013; TCEQ, 2014a,b). When expressed as air concentrations associated with an additional lifetime cancer risk of 1 in 1,000,000, these values equate to 0.3 ng/m^3 (for workers exposed for 40 years; DECOS, 2016), and 0.034 and 0.4 ng/m^3 (for the general population exposed for 70 years; ECHA, 2013; TCEQ, 2014a,b). EMA experts also set an inhalation PDE for Cr(VI) of 10 ng/day, associated with an increased cancer risk of 1 in 100,000 [implying that a concentration of 0.5 ng/m^3 (assuming an inhalation rate of 20 m^3/day) would produce an additional lifetime cancer risk of 1 in 100,000] (EMA, 2008). The most precautionary benchmark was established from epidemiological data on the basis of an excess lifetime lung cancer mortality risk of 2.9 x 10^{-2} per µg Cr(VI)/m^3 (ECHA, 2013).

Following oral exposure, Cr(VI) has also been considered to lack a carcinogenic threshold. Extrapolation of the identified cancer HCVs for this route yields doses ranging from 0.2-1.25 ng/kg bw/day which would be associated with excess cancer risk values of 1 in 1,000,000 (ECHA, 2013; SCHER, 2015; US EPA, 2010). In contrast, other experts have considered that a threshold-based MoA is responsible for the toxic effects seen following oral exposure. Health Canada derived a TDI of 4 µg/kg bw (HC, 2015), and TCEQ is of a similar

opinion, deriving an RfD of 3 µg/kg bw/day for oral Cr(VI) (TCEQ, 2016). An RfD of 6 µg/kg bw/day has been derived by other experts in a detailed publication (Thompson *et al.*, 2014), and RIVM's TDI is 5 µg/kg bw (RIVM, 2001). These values are all very similar to an RfD derived by the US EPA for non-cancer effects (3 µg/kg bw/day) (US EPA, 1998b).

The WHO has set a provisional drinking water guideline of 50 µg/L for chromium (total Cr(III) and VI). This value is considered to be unlikely to give rise to significant risks to health, though the WHO noted the uncertainty surrounding the applicability of this figure due to the genotoxic carcinogenicity of Cr(VI) by the inhalation route. It was considered that the limit would be retained as a provisional guideline value until additional information enabling re-evaluation of chromium became available (WHO, 2003). For an adult weighing 70 kg and drinking 2 L/day of water containing chromium at this guideline limit, the chromium dose would be about 1.4 µg/kg bw/day.

Besides their other systemic effects, Cr(VI) compounds are considered to be sensitisers to the skin and respiratory tract. Cr(III) is also associated with contact allergy (ATSDR, 2012; HPA, 2007).

The valency of the chromium extractable is not known, and so it is assumed as a worst case that is could theoretically all be Cr(VI). Note, though, that Cr(VI) is unstable in the body and reduced to Cr(III) *in vivo* by a variety of reducing agents (IPCS, 2009, 2013). Such reduction to less-toxic forms is expected to be very efficient at low exposures (EMA, 2008).

The critical toxicity concern from Cr(VI) exposure is genotoxic carcinogenicity. As such, the most precautionary cancer benchmark, derived by ECHA, is selected as the critical POD from which to derive a cancer TI. According to ECHA's calculations, inhalation at 0.034 ng/m^3 continuously for 70 years would be associated with a maximum cancer risk of 1 in 1,000,000). Assuming a 70-kg adult inhales air at 20 m^3/day (ECHA defaults), this corresponds to a dose level of 0.0099 ng/kg bw/day. Regulators and other experts generally view cancer risks of 1-10 in 1 million as negligible/tolerable. For example, ICH M7 uses a cancer risk benchmark of 1 in 100,000. A cancer risk of 1 in 100,000 would thus be associated with continuous lifetime exposure at 0.099 ng/kg bw/day, and this is adopted here as a cancer TI.

Consumer population	Cancer TI for Cr(VI) (ng/kg bw/day)	Maximum exposure to chromium (ng/kg bw/day)	Margin of Safety
Adults	0.099	5.2	0.02

As shown in the table above, an excess cancer risk outside of acceptable margins would be posed by the hypothetical exposure to Cr(VI) resulting from vaping 30 mL e-liquid per day. Therefore, a more refined estimate of cancer risk is necessary. An estimate of maximum cancer risk may be derived assuming that the main driver of lifetime cancer risk is cumulative exposure. The TI and the tolerable exposure values defined by Expert Groups correspond to daily exposures to Cr(VI) for a lifetime. However, a more realistic period of time to consider in this risk assessment is 40 years. If a cumulative exposure of 17.7 µg[64] over 70 years is associated with a cancer risk of 1 in 1,000,000, then exposure to at 5.2 ng/kg bw/day for 40 years (total 4555 µg for a 60-kg adult) would be associated with an excess cancer risk of

[64] 0.0099 ng/kg bw/day * (70 years x 365 days/year) = 253 ng/kg bw * 70 kg bw = 17.7 µg.

about 2.5 in 10,000. This exposure still falls outside of the widely-accepted margins of tolerable cancer risks.

However, it is highly unlikely that the metal is present as Cr(VI); any chromium would likely be present in the zero-valence state and, if any Cr(VI) was present, is likely to be rapidly reduced to Cr(III). It is therefore appropriate to consider the PDE derived by the ICH for Cr(III) and Cr(0)[65]. The inhalation PDE of 2.9 µg/day (rounded to 3 µg/day, equivalent to 0.06 µg/kg bw/day for a 50-kg individual), estimated by focussing on the toxicity of Cr(III) and Cr(0), may therefore be adopted as a more realistic TI for chromium in this case.

Consumer population	TI for Cr(III) and Cr(0) (ng/kg bw/day)	Maximum exposure to chromium (ng/kg bw/day)	Margin of Safety
Adults	60	5.2	11.5

As shown in the table above, the maximum daily exposure to chromium being considered in this assessment is 11.5-fold lower than this benchmark. It can therefore be concluded that exposure to chromium (in its Cr(III) and Cr(0) valences) is highly unlikely to pose any significant health risk to consumers.

With regards to the potential risk of Cr(VI) causing respiratory sensitisation, it is reassuring to note that the assessed exposure (0.312 µg/day) is significantly below the health-precautionary Qualification Threshold of 5 µg/day for sensitisers in orally-inhaled and nasal drug products (OINDPs) set by experts scientists of the Product Quality Research Institute (PQRI) (see Appendix V). On that basis, chromium is unlikely to pose a significant risk of inducing sensitisation. The possibility of a rare case of elicitation of sensitisation in consumers already sensitised by previous exposures via other sources (this generally manifests at lower doses) cannot be entirely excluded.

Residual uncertainty remains over the carcinogenic potential of Cr(VI) as a potential leachable. However, if some small proportion is present as a leachable, e.g. up to 4%, the cancer risks of a 40-year exposure would be considered to be within acceptable limits (i.e. less than 1 in 100,000). As emphasised by the ICH in regard to pharmaceuticals, any chromium leaching from container closure systems is highly unlikely to be in this unstable Cr(VI) valence state. Therefore, overall, it can be concluded that exposure to chromium is unlikely to pose any significant health risk to consumers.

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COPPER

The ICP-MS analysis detected copper (CAS RN 7440-50-8) in the container (natural and translucent black), inner closure (natural and black), the natural and translucent black PP and LDPE nozzle components. Consumers vaping 30 mL e-liquid per day by filling up their ENDS using the 10 mL CCS (with the natural container, natural inner closure and natural LDPE nozzle) may therefore be exposed at up to 0.76 µg/day, equivalent to approximately 12.7 ng/kg bw/day for a 60-kg individual.

Copper is an essential trace element for humans, playing a vital role in some critical enzyme systems and closely linked with normal haematopoiesis and cellular metabolism.

Short-term ingestion of copper may result in adverse effects on the gastrointestinal tract (nausea, pain and vomiting, diarrhoea). Long-term effects are less well documented; current evidence indicates that, in the general population, chronic exposure to very high levels of copper may lead to effects in the liver and kidney (EFSA, 2017; Health Canada, 2018; ICH, 2019; Nordberg *et al.*, 2018).

According to the European Food Safety Authority, mean copper intakes in eight EU countries range from 1.27-1.67 mg/day in adult men and 1.15-1.44 mg/day in non-pregnant adult women. Based on observed intakes and the fact that copper balance was reported at about 1.6 mg/day in men, EFSA proposed an adequate intake (AI) values of 1.6 mg/day for men and 1.5 mg/day for women. Specific AIs were also set for infants and children (EFSA, 2017).

Health Canada has recently proposed a MAC of 2 mg/L in the drinking water, equivalent to 4 mg/day assuming an average daily water consumption of 2 L. The MAC is considered protective of both short-term (i.e. gastrointestinal effects) and long-term (potential liver and kidney effects) toxicity (Health Canada, 2018).

In the absence of any adequate inhalation toxicity studies, ICH experts based their inhalation PDE on the analogous oral benchmark. The critical PoD was the NOEL of 1000 ppm reported in a 13-week dietary study with cupric sulphate pentahydrate in rats (higher dietary concentrations produced toxicity of the liver, kidney and forestomach). ICH applied a total MF of 250 to the NOEL (equivalent to 17 mg Cu/kg bw/day) to generate the oral PDE of 3400 µg/day for a 50-kg patient. An additional factor of 100 was applied to account for the oral bioavailability of copper and its inorganic salts. The inhalation PDE was therefore 34 µg/day, equivalent to 0.68 µg/kg bw/day (ICH, 2019).

The ICH (inhalation) PDE can therefore be adopted as the TI for copper.

Consumer population	TI for copper (ng/kg bw/day)	Maximum exposure to copper (ng/kg bw/day)	Margin of Safety
Adults	680	12.7	54

As shown in the table above, the maximum exposure to copper resulting from vaping 30 mL e-liquid per day is 54-fold lower than the TI. It can therefore be confidently concluded that exposure to copper is highly unlikely to pose any significant health risk to consumers.

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LITHIUM

The ICP-MS analysis detected lithium (CAS RN 7439-93-2) in the container (natural and translucent black) and LDPE nozzle (natural and translucent black) components. Consumers vaping 30 mL e-liquid per day by filling up their ENDS with the 10 mL CCS (with the natural container and natural LDPE nozzle) may therefore be exposed at up to 321 ng/day, equivalent to approximately 5.36 ng/kg bw/day for a 60-kg individual.

Lithium is a common metal that is present in plant and animal tissues. Because of its similarity to sodium and potassium, lithium easily crosses all biological barriers, meaning that its shows almost complete absorption and a uniform distribution in body fluids. Not being protein bound, lithium is rapidly eliminated by the kidneys, with almost no tissue accumulation (Nordberg *et al.*, 2018). There are extensive human data regarding the administration of lithium salts in the treatment of various psychiatric disorders (ICH, 2019). As the therapeutic index of lithium is very low, side effects are quite common and mainly consist of a reduced urinary concentration capacity, hypothyroidism, and neurotoxic effects (Nordberg *et al.*, 2018).

According to the ICH, "no significant effects" were reported in rabbits exposed to lithium chloride at 0.6 and 1.9 mg/m^3 for 6 hours/day, 5 days/week for 4-8 weeks. Consequently, the highest tested concentration was used as the PoD in derivation of the ICH inhalation PDE. The NOAEC was extrapolated to a continuous concentration of 0.34 mg/m^3 and subsequently converted to a systemic dose of 122 µg/kg bw/day[66]. UFs of 2.5, 10 and 10 were then applied to account for interspecies differences (rabbit to human), interindividual variation and the short-term exposure, yielding an inhalation PDE of 0.5 µg/kg bw/day (ICH, 2019).

[66] (1.9 mg/m^3 x 6/24 x 5/7 x 1.44 m^3/day) / 4 kg bw.

In addition, ICH experts derived its oral PDE of 560 µg/day considering the clinical posology of lithium. The lowest single oral dose of lithium chloride was evidently 300 mg, equivalent to 56 mg lithium, and it was noted that toxic effects can occur even at therapeutic doses. Consequently, a total MF of 100 was applied to yield the oral PDE (ICH, 2019).

The ICH (inhalation) PDE can be adopted as the TI for lithium.

Consumer population	TI for lithium (ng/kg bw/day)	Maximum exposure to lithium (ng/kg bw/day)	Margin of Safety
Adults	500	5.36	93.3

As shown in the table above, the maximum exposure to lithium resulting from vaping 30 mL e-liquid per day is 93-fold lower than the TI. It can therefore be confidently concluded that exposure to lithium is highly unlikely to pose any significant health risk to consumers.

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NICKEL

The ICP-MS analysis detected nickel (CAS RN 7440-02-0) in the container (translucent black), inner closure (natural), PP nozzle (natural and translucent black) and LDPE nozzle (translucent black) components. Consumers vaping 30 mL e-liquid per day by filling up their ENDS using the 10 mL CCS (with translucent black container, natural inner closure and translucent black LDPE nozzle) may therefore be exposed at up to 0.72 µg/day, equivalent to approximately 12 ng/kg bw/day for a 60-kg individual.

ICH experts have noted that the ingestion of large doses of nickel may cause stomach pain, depression of body weight and adverse effects on blood and kidneys. Humans generally become sensitised to nickel only after prolonged contact with the skin (ICH, 2019). Nickel is considered to be genotoxic but not mutagenic. There is no indication of carcinogenicity after oral administration of nickel salts but there is sufficient evidence in humans for the role of inhaled mixtures including nickel compounds and nickel metal in causing cancers of the lung, nasal cavity and paranasal sinuses, and nickel compounds are considered carcinogenic to humans (Group 1) by IARC (IARC, 2012).

Nickel oxide was not carcinogenic in chronic inhalation studies with hamsters (Wehner *et al*., 1984) or mice (NTP, 2006). There was some evidence of carcinogenicity when this substance was tested in rats by inhalation (NTP, 2006), though this was not replicated in a later investigation with metallic nickel (Oller *et al*., 2008). In deriving a PDE for nickel by the inhalation route, ICH considered that the MF approach was acceptable “because the forms

and levels likely to be in inhalation drug products have not shown evidence of carcinogenicity" and utilised the NOAEC of 0.5 mg Ni/m^3 from the NTP rat study as the critical PoD. After extrapolating to a continuous exposure concentration (6 hr/24 hr x 5 days/7 days), a tolerable daily dose of 0.06 mg/kg bw/day (rats) was calculated, assuming rat body weight and inhalation rate values of 0.425 kg and 0.29 m^3/day, respectively. A total MF of 500 was applied to yield the inhalation PDE of 0.12 µg/kg bw/day, equivalent to (when rounded down) approximately 5 µg/day for a 50-kg adult (ICH, 2019).

Respective PDEs of 200 and 20 µg/day (rounded down from 220 and 22 µg/day, respectively) were established for the oral and parenteral routes, based on a 2-year oral carcinogenicity investigation with nickel sulphate hexahydrate in rats (ICH, 2019). In a previous draft, ICH had selected sensitisation in humans as being critical, deriving higher oral and parenteral PDEs of 600 and 60 µg/day, respectively, based on the observation that oral challenge with 12 µg Ni/kg bw can induce dermatitis in nickel sensitized individuals (ICH, 2013).

The allergenic potential of nickel is well-known. Indeed, a WHO expert group noted that the incidence of sensitisation following workplace exposure to airborne nickel is well established, although there is no evidence pertaining to such effects in the general population. Nevertheless, and in apparent contrast to the ICH, the WHO experts considered [non-threshold] carcinogenicity as the key criterion for assessing the risk of nickel exposure. An inhalation unit risk of 3.8×10^{-4} per µg Ni/m^3 was calculated based on the observation of lung cancer in an occupational cohort study in Norway, corresponding to an excess lifetime lung cancer risk of 1 in 100,000 following exposure at 0.025 µg/m^3 (WHO, 2000).

Similarly, the Texas Commission on Environmental Quality (TCEQ) considered nickel compounds as a group to be carcinogenic to humans, with metallic nickel having at most "suggestive evidence of carcinogenic potential". Epidemiological lung cancer data was used to calculate a long-term health concentration of 0.059 µg/m^3 for nickel (and inorganic nickel compounds) as being associated with a 1 in 100,000 excess lung cancer risk, based on a cancer unit risk factor (URF) of 0.00017 per µg/m^3 (TCEQ, 2011).

In a recent assessment of nickel and its inorganic compounds by ECHA experts, respective occupational exposure limits (OELs) of 0.005 and 0.03 mg/m^3 were proposed for respirable and inhalable dust; the derivation of the former utilised a NOAEC of 0.03 mg Ni/m^3 from a chronic rat inhalation study with nickel sulphate (lack of inflammatory effects and lung tumours), while the latter value was calculated based on occupational lung cancer data (ECHA, 2018).

The ICH inhalation PDE of 5 µg/day would seem to be an appropriate value for the current assessment. It is therefore adopted as the TI for nickel.

Consumer population	TI for nickel (ng/kg bw/day)	Maximum exposure to nickel (ng/kg bw/day)	Margin of Safety
Adults	120	12	10

As shown in the table above, the exposure being assessed here is 10-fold lower than the TI. On this basis, the estimated exposure to nickel is unlikely to pose a significant health risk to consumers.

It is useful to consider the WHO and TCEQ cancer-driven limits in order to provide further context and to facilitate quantification of any cancer potential that may be associated with exposure to nickel at the detected level. These limits equate to inhaled doses of 0.5-1.2 µg/day, which are slightly more health-precautionary than the ICH benchmark. Cancer risk is largely driven by cumulative exposure. If exposure to nickel at 0.5-1.2 µg/day for 70 years is associated with a maximum cancer risk of 1 in 100,000, then exposure at 0.72 µg/day for 40 years implies a lifetime cancer risk value in the order 0.3-0.8 in 100,000 and is therefore generally considered to be of no practical significance.

Although nickel is a well-established skin sensitiser and cases of allergic contact dermatitis to e-cigarette devices have been reported in the literature (Shim and Kosztyuova, 2018), the respiratory sensitisation potential of this substance is comparatively poorly studied, and a quantitative assessment is not possible. However, it is reassuring to note that the assessed exposure (0.72 µg/day) is significantly below the health-precautionary Qualification Threshold of 5 µg/day for sensitisers in orally-inhaled and nasal drug products (OINDPs) set by expert scientists of the Product Quality Research Institute (PQRI) (see Appendix V). On that basis, any nickel from the CCS is unlikely to pose a significant risk of inducing sensitisation. The possibility of a rare case of elicitation of sensitisation in consumers already sensitised by previous exposures via other sources (this generally manifests at lower doses) cannot be entirely excluded.

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LEAD

The ICP-MS analysis detected lead (CAS RN 7439-92-1) in the inner closure (natural) at 4.52 ng/g. Consumers vaping 30 mL e-liquid per day may therefore be exposed at up to 15.05 ng/day, equivalent to approximately 0.25 ng/kg bw/day for a 60-kg individual.

Lead is an accumulative toxin and can cause neurological, reproductive, developmental, immune, cardiovascular and renal toxicity in humans and laboratory animals. In general, the foetus and young child are more susceptible to lead toxicity than are adults (ATSDR, 2019; ICH, 2019).

ICH has derived a PDE of 5 µg/day for lead, irrespective of the exposure route. This was sourced from the application of a standard US EPA pharmacokinetic model indicating (assuming 100% absorption and no other sources of lead) that an intake by any route of exposure of 5 µg Pb/day by a child throughout its first 7 years of life will produce a blood lead level of 1-2 µg/dL (US EPA, 2009). Since the data are based on blood levels, the lead PDEs derived by ICH are the same regardless of the route of administration (ICH, 2019).

The ICH PDE of 5 µg/day (equivalent to 0.1 µg/kg bw/day for a 50-kg individual) can be adopted as a TI for lead.

Consumer population	TI for lead (ng/kg bw/day)	Maximum exposure to lead (ng/kg bw/day)	Margin of Safety
Adults	100	0.25	400

As shown in the table above, the exposure being assessed here is 400-fold lower than the TI. On this basis, the estimated exposure to lead is unlikely to pose a significant health risk to consumers.

References

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ANTIMONY

The ICP-MS analysis detected antimony (CAS RN 7440-36-0) in the black inner closure component at 2.71 ng/g. Consumers vaping 30 mL e-liquid per day by filling up their ENDS with the 10 mL CCS may therefore be exposed at up to 9.03 ng/day, equivalent to approximately 0.15 ng/kg bw/day for a 60-kg individual.

Antimony is nutritionally not essential, and no metabolic function is known. In humans and other animals, the gastrointestinal tract appears to be the primary target organ after ingestion, with reported effects including irritation, diarrhoea and vomiting. In subchronic studies in rats, lower body weight and adverse liver findings were the most sensitive endpoints. Inhalation of high levels over a long period can cause adverse respiratory effects in both humans and laboratory animals (ICH, 2019).

Various antimony compounds have tested negative for mutagenicity in both bacterial and mammalian cells (Amalgamated Metal Corporation Plc *et al.*, 2019; NTP, 1992). There are indications that antimony (III) may be positive for clastogenicity *in vitro*, though these findings are generally not replicated *in vivo* (ICH, 2019; WHO, 2003). A recent assessment of a group of 11 antimony-containing substances by the Canadian health authorities concluded that "overall, there is [only] a low concern for genotoxicity for the antimony substances in the group" (ECHC/HC, 2018). Given also that antimony has been allocated a TDI figure by experts from the Danish EPA (2015), Dutch authorities (RIVM, 2009) and WHO (2003), clearly this element is not considered to be a genotoxic carcinogen. It is therefore appropriate to assess this element as a threshold toxin.

Regarding the potential for antimony compounds to induce sensitisation, a review by Danish experts identified a single well-conducted guinea pig maximization test that demonstrated that antimony trioxide has no skin sensitising properties. None of the studies in humans were considered reliable, and no data on other inorganic antimony compounds were located (Danish EPA, 2015). The REACH dossier on antimony did not identify any additional reliable sensitisation studies (Amalgamated Metal Corporation Plc *et al.*, 2019). Based on the available data, it is considered unlikely that exposure to this element would induce any sensitisation effects in consumers.

According to ICH experts, lung effects (increased lung weights) have been observed consistently in rats following subchronic and chronic inhalation to antimony compounds. Using a key NOAEC[67] of 0.9 mg/m^3 identified in a 13-week inhalation study in which rats were exposed to antimony trioxide dust (6 hours/day, 5 days/week), the ICH derived its inhalation PDE for antimony. The NOAEC was extrapolated to a continuous concentration of 0.16 mg/m^3 and converted to a systemic dose of 0.11 mg/kg bw/day[68] before the application of UFs of 5, 10 and 5 to take account of interspecies differences (rat to human), interindividual variation, and the subchronic study duration. This yielded a PDE of 0.4 µg/kg bw/day, or 22 µg/day for a 50-kg individual (ICH, 2019).

The ICH (inhalation) PDE can be adopted as the TI for antimony.

Consumer population	TI for antimony (ng/kg bw/day)	Maximum exposure to antimony (ng/kg bw/day)	Margin of Safety
Adults	400	0.15	2667

As shown in the table above, the maximum exposure to antimony resulting from vaping 30 mL e-liquid per day is 2667-fold lower than the TI. It can therefore be confidently concluded that exposure to antimony is highly unlikely to pose any significant health risk to consumers.

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[67] The key NOAEC was originally defined as 1.08 mg/m^3, but a value of 0.9 mg/m^3 was subsequently used as the PoD by the ICH (2019) for unknown reasons.

[68] 0.9 mg/m^3 x 6/24 x 5/7 x 0.29 m^3/day / 0.425 kg

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WHO (2003). World Health Organization. Guidelines for drinking-water quality. Antimony.
http://www.who.int/water_sanitation_health/dwq/chemicals/antimonysum.pdf

SELENIUM

The ICP-MS analysis detected selenium (CAS RN 7782-49-2) in the solid black and translucent black containers and the translucent black LDPE nozzle. Consumers vaping 30 mL e-liquid per day by filling up their ENDS with the 10 mL CCS (with translucent black container and translucent black LDPE nozzle) may therefore be exposed at up to 353 ng/day, equivalent to approximately 5.9 ng/kg bw/day for a 60-kg individual.

Selenium is an essential element in humans. It is considered to have anti-cancer properties at low doses but may be genotoxic and carcinogenic at higher doses. In humans, chronic oral exposure is associated with liver toxicity, neurological effects and changes to the nails, skin and hair (WHO, 2006).

ICH has derived parenteral and inhalation PDEs for selenium of 80 and 130 µg/day (rounded down from 85 and 135 µg/day, respectively). ICH utilised the US Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL) of 0.2 mg/m^3 when deriving its inhalation PDE for the element[69]. This was adjusted to an equivalent figure for continuous exposure of 0.048 mg/m^3 (0.2 mg/m^3 x 8 hr/24 hr x 5 days/7 days), equating to a daily dose of 0.027 mg/kg bw/day (0.048 mg/m^3 x 28.8 m^3/50 kg). A total MF of 10 was applied to yield the inhalation PDE of 2.7 µg/kg bw/day, equivalent to 135 µg/day for a 50-kg adult. For the parenteral PDE, the key study was a chronic gavage study with selenium sulphide in rats (NTP, 1980); an MF of 500, with an additional factor of 2 to account for bioavailability differences[70], was applied to yield the parenteral PDE of 85 µg/day for a 50-kg adult (ICH, 2019).

The ICH (inhalation) PDE can be adopted as the TI for selenium.

Consumer population	TI for selenium (ng/kg bw/day)	Maximum exposure to selenium (ng/kg bw/day)	Margin of Safety
Adults	2700	5.9	458

As shown in the table above, the maximum exposure to selenium resulting from vaping 30 mL e-liquid per day is 458-fold lower than the TI. It can therefore be confidently concluded that exposure to selenium is highly unlikely to pose any significant health risk to consumers.

[69] These PEL limits are published by the US Department of Labor.

[70] Based on oral absorption values of >80% for various selenium compounds (ATSDR, 2003)

References

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VANADIUM

The ICP-MS analysis detected vanadium (CAS RN 7440-62-2) in the black inner closure component at 4.52 ng/g. Consumers vaping 30 mL e-liquid per day may therefore be exposed at up to 15.05 ng/day, equivalent to approximately 0.25 ng/kg bw/day for a 60-kg individual.

Vanadium is ubiquitous in the human body, although an essential biological role has not been established. It is genotoxic, but not mutagenic, and vanadium pentoxide is classified as a possible human carcinogen, with carcinogenic effects reported in inhalation studies in rats. After oral administration, the gastrointestinal tract, cardiovascular, and haematological systems were the primary targets of toxicity (ICH, 2019).

An ICH oral PDE was derived on the basis of a 12-week study in volunteers given vanadium sulphate or ammonium vanadyl tartrate by capsule (ATSDR, 2012). ICH applied a total MF of 50 to the identified NOAEL of 0.12 mg/kg bw/day (expressed as vanadium), to derive an oral PDE of 100 µg/day (rounded down from 120 µg/day) for a 50-kg adult (ICH, 2019).

EMA experts set a comparable oral PDE figure of 250 µg/day for vanadium. The derivation was based on the identified human no-observed-effect level (NOEL) of 0.5 mg/kg/day with due consideration of data on dietary intake of this element (0.3 µg/kg bw/day) as well as US EPA RfDs for a range of vanadium compounds (1-20 µg V/kg bw/day) (EMA, 2008).

Previously, Dutch experts derived a provisional TDI of 2 µg/kg bw/day [120 µg/day] for vanadium based on developmental toxicity in rats (RIVM, 1998).

Although ICH experts identified a 2-year inhalation toxicity study in rats, the results (increased incidence of tumours even at the lowest tested concentration) were not considered to be relevant for the derivation of an inhalation PDE as the test material, vanadium pentoxide, was considered a caustic substance. Instead, the ICH calculated the

inhalation PDE by dividing the oral PDE by a factor of 100, yielding 0.024 µg/kg bw/day, or 1.2 µg/day for a 50-kg individual (ICH, 2019).

It is therefore appropriate to consider the ICH inhalation PDE as the TI for vanadium.

Consumer population	TI for vanadium (ng/kg bw/day)	Maximum exposure to vanadium (ng/kg bw/day)	Margin of Safety
Adults	24	0.25	96

As shown in the table above, the maximum exposure to vanadium resulting from vaping 30 mL e-liquid per day is almost 100-fold lower than the TI. It can therefore be confidently concluded that exposure to vanadium is highly unlikely to pose any significant health risk to consumers.

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CONCLUSION

The analytical studies conducted on the PET containers (natural, solid black and translucent black), inner closures (natural and solid black), LDPE nozzle (natural and translucent black) and PP nozzle (natural and translucent black) components of the CCS detected a number of organic and inorganic extractables (potential leachables) to which consumers could potentially be exposed via vaping. From the results of the HS-GC-FID/MS, GC-FID/MS, LC-DAD/MS and ICP-MS analyses, worst-case consumer exposures were estimated, and possible health risks were assessed.

By comparing the highest anticipated concentration of these potential leachables within each inhalation with tolerable concentrations (TCs) of a potent respiratory tract irritant (formaldehyde), it was concluded that respiratory tract irritation is not of practical concern with respect to the identified extractable substances.

For chemically-identified extractables lacking mutagenic character (i.e. threshold toxins), the health risk assessment was based on key NOAELs from appropriate high-quality repeated dose toxicity studies where possible, supported by Expert Group derivations of tolerable exposure figures such as permitted daily exposures (PDEs), and health risk evaluations. Where necessary and appropriate, toxicity data on structurally-similar analogues were also used (in a read-across approach). ISO 10993-17 and ICH principles were followed, and the maximum worst-case exposures were compared with tolerable intakes (TIs) derived for each extractable.

Based on laboratory studies, Expert Group conclusions and/or Toxtree structure-activity relationships (SAR) the majority of the identified extractables were concluded to lack mutagenic potential and were thus assessed as threshold toxins. Margins of Safety were determined to be greater than unity for each extractable (or group of extractables), thus demonstrating tolerability.

As chromium (Cr) was identified in the ICP-MS analysis, the toxicological profiles of the most common valence states (Cr(III) and elemental chromium, Cr(0)) were assessed. The maximum daily exposure to Cr was reassuringly >10-fold lower than the inhalation PDE derived for Cr by the ICH. Furthermore, as a highly health-precautionary measure, the toxicology of Cr(VI), a carcinogenic species, was also considered. By comparing the extreme worst-case estimates of exposure (assuming 100% of the extracted Cr to be in the hexavalent state) with tolerable intake values derived by Expert Groups, the resulting excess cancer risks were not determined to be within acceptable levels. However, tolerable cancer risks were estimated if up to 4% of the extracted Cr were in the hexavalent form. In reality, any Cr would likely be present in the zero-valence state, and any Cr(VI) present is likely to be rapidly reduced to Cr(III) and potential exposures to Cr(VI) are therefore likely to be far below 4% of the identified Cr. Overall, therefore, the potential exposure to Cr is unlikely to pose any significant health risks to consumers.

A respiratory sensitisation potential is acknowledged for both chromium and nickel. From the analysis, the calculated exposures were determined to be very low (0.3 µg/day and 0.7 ng/day, respectively). Although it is not possible to confidently determine a safe benchmark for these sensitisers, based on the very low estimates of exposure these two extractables are highly unlikely to pose any significant risk of inducting new cases of sensitisation. This is reassuring. However, it should be noted that the possibility of an occasional reaction in highly-sensitive individuals who have already been sensitised to these substances by other exposures, cannot be entirely excluded.

Overall it was concluded that the potential exposure to these extractables is unlikely to pose any significant health risks to consumers vaping e-liquid at 30 mL/day (a worst-case scenario).

Moreover, in reality, any potential leaching of these organic and inorganic species from the CCS into the e-liquid is likely to be far less extensive than has been observed in these exhaustive extractables studies (under exaggerated conditions). As such, far lower exposures would be anticipated in a leachables study and/or in real use than have been estimated in this extractables assessment, and thus even more reassuring Margins of Safety would be established.

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Appendix I: The TRACE database and databank
Bibra toxicology advice & consulting Ltd

TRACE includes information from peer-reviewed toxicology and nutrition journals as well as secondary sources and websites. In addition to primary literature on the health effects of chemicals, TRACE covers official publications and evaluations issued by authoritative Expert groups, including:

- WHO/IPCS reports and evaluations (including CICADs and EHCs, and IARC, JECFA and JMPR monographs), and the WHO Air Quality and Drinking-Water Quality Guidelines
- OECD SIDS dossiers/SIARS and CoCAMs
- IUCLID data sets
- EU Risk Assessment Reports
- EU expert committee opinions (including EU scientific committees, and EFSA scientific panels) and other reports from EU agencies and institutes etc. Includes ECHA, RAC, SCHEER, SCENIHR, SCCS, SCOEL, ECVAM, EMA, ICCG, SCF and CPS&Q)
- ECETOC, HERA, Council of Europe and other pan-European programmes
- UK government agency (including Defra, EA, FSA, DoH, HSE, HPA, PSD and VMD) and advisory committee (e.g. COT, COM, COC, ACNFP, SACN, ACP, ACAF, VPC, VRC and ACRE) reports and evaluations
- Opinions from other UK organisations such as the Royal Society
- US agency reports and evaluations (EPA, ATSDR, FDA, NTP, OSHA, NCEA, CFSAN, CERHR, NIEHS, CDC, OEHHA and ACGIH)
- Health Canada evaluations
- German BUA, DFG, BG Chemie and BfR reports and monographs
- Dutch RIVM reports and Gezondheidsraad opinions (including those from its various committees such as DECOS)
- Danish EPA reviews
- Reports and other information provided by Swedish governmental organisations, including the National Food Administration and the Swedish Chemicals Agency
- Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals
- Australian agency reviews including NICNAS Priority Existing Chemical Assessments, APVMA reports and (jointly with New Zealand) FSANZ assessments
- Japanese Chemical Industry Ecology-Toxicology & Information Center reports
- CIR, RIFM, FEMA and other specialist industry groups
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)
- International Standards Organization (ISO)
- Bibra Toxicity Profiles

Appendix II: TOXNET network
US National Library of Medicine

TOXNET – Databases and databanks on toxicology, hazardous chemicals, environmental health, and toxic releases, which includes:

- Toxline – Toxicology literature online
- DART – Developmental Toxicology literature
- ChemIDplus – Chemical identification/Dictionary
- HSDB – Hazardous Substances Data Bank
- CCRIS – Chemical Carcinogenesis Research Information System
- CPDB – Carcinogenic Potency Database
- GENETOX – Genetic Toxicology Data
- IRIS – Integrated Risk Information System
- ITER – International Toxicity Estimates for Risk
- LactMed – Drugs and Lactation Database
- TRI – Toxics Release Inventory
- TOXMAP – Environmental Health e-maps
- Haz-Map – Occupational Exposure/Toxicology
- CTD – Comparative Toxicogenomics Database
- Household Products Database
- ALTBIB – Resources on Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing

Appendix III: eChemPortal
The global portal to information on chemical substances
Organisation for Economic Co-operation and Development

Databases currently participating in eChemPortal:

- ACToR - U.S. EPA Aggregated Computational Toxicology Resource
- AGRITOX - Base de données sur les substances actives phytopharmaceutiques
- APVMA-CR - The Australian Pesticides and Veterinary Medicines Authority (APVMA) database of completed chemical reviews
- CCR - Canadian Categorization Results
- CESAR - Canada's Existing Substances Assessment Repository
- Combined Exposures - Collection of Case Studies on Risk Assessments of Combined Exposures to Multiple Chemicals
- ECHA C&L inventory - Public Classification and Labelling (C&L) Inventory according to the European Union (EU) CLP Regulation (EC) No 1272/2008
- ECHA CHEM - European Chemicals Agency's Dissemination portal with information on chemical substances registered under REACH
- EFSA Open Food Tox - Chemical Hazards Database of the European Food Safety Authority
- EnviChem - Data Bank of Environmental Properties of Chemicals
- EPA HHBP - EPA Human Health Benchmarks for Pesticides
- EPA OPPALB - EPA Office of Pesticide Programs' Aquatic Life Benchmarks
- GDL- Gefahrstoffdatenbank [Dangerous Substances Databank] der Länder (Germany)
- GHS-J - GHS Classification Results by the Japanese Government
- GSBL - Joint Substance Data Pool of the German Federal Government and the German Federal States
- HPVIS - High Production Volume Information System (US EPA)
- HSDB - Hazardous Substances Data Bank
- HSNO CCID - New Zealand Hazardous Substances and New Organisms Chemical Classification Information Database
- IGS - Public Informationssystem für gefährliche Stoffe (Germany)
- INCHEM - Chemical Safety Information from Intergovernmental Organizations
- INERIS-PSC - INERIS-Portail Substances Chimiques
- IPCHEM - Information Platform for Chemical Monitoring
- J-CHECK - Japan CHEmicals Collaborative Knowledge database
- JECDB- Japan Existing Chemical Data Base
- NICNAS IMAP - Australia's National Industrial Chemicals Notification and Assessment Scheme's Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework
- NICNAS Other - Australian National Industrial Chemicals Notification and Assessment Scheme assessments of existing chemicals other than Priority Existing Chemical assessments
- NICNAS PEC - Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) Priority Existing Chemical Assessment Reports
- OECD HPV - Organisation for Economic Cooperation and Development Existing Chemicals Database, High Production Volume
- OECD SIDS IUCLID - OECD Existing Chemicals Screening Information Data Sets International Uniform Chemical Information Database
- SIDS UNEP - OECD Initial Assessment Reports for HPV Chemicals including Screening Information Data Sets as maintained by United Nations Environment Programme
- SPIN - Substances in Preparations In the Nordic countries
- UK CCRMP Outputs - UK Coordinated Chemicals Risk Management Programme Publications
- US EPA IRIS - United States Environmental Protection Agency Integrated Risk Information System
- US EPA SRS - United States Environmental Protection Agency Substance Registry Services

Appendix IV: ECHA's Information on Chemicals database

European Chemicals Agency

ECHA's database provides information on chemicals manufactured/imported in Europe. It covers their hazardous properties, classification and labelling, and information on how to use them safely; some data are provided by third parties.[71] The database includes the following resources (as of June 2016):

REACH (Regulation (EC) No 1907/2006)

- Registered substances (REACH registration dossiers) and pre-registered substances
- Annex III Inventory – an inventory of low-tonnage substances likely to meet Annex III criteria
- EC Inventory (including EINECS (European Inventory of Existing Commercial chemical Substances), ELINCS (European List of Notified Chemical Substances) and NLP (No-Longer Polymers))
- Dossier evaluation decisions
- Testing proposal consultations
- Substance evaluation – CoRAP (the Community Rolling Action Plan)
- Candidate List of Substances of Very High Concern (SVHCs) for authorisation
- Restricted substances
- Public Activities Coordination Tool (PACT)

CLP (Regulation (EC) No 1272/2008)

- The C&L Inventory – Classification and labelling information on notified and registered substances
- Chemicals listed in Annex VI of the CLP (and subject to harmonised classification and labelling)

Biocides (as regulated by the BPR, Regulation (EU) 528/2012)

- Biocidal active substances
- Biocidal products
- Article 95 List of biocidal active substances and suppliers

Prior Informed Consent (Regulation (EU) 649/2012)

- Chemicals subject to PIC
- Export and import notifications
- Explicit consent and waivers

Information from previous chemicals legislation

- Risk assessment reports performed under Council Regulation (EEC) No 793/93[72]
- Annex XV transitional Reports
- Persistent, bioaccumulative and toxic (PBT)/very persistent, very bioaccumulative (vPvB) assessments under previous EU chemicals legislation

[71] Information on Registered Substances … comes from registration dossiers which have been assigned a registration number. The assignment of a registration number does however not guarantee that the information in the dossier is correct or that the dossier is compliant with Regulation (EC) No 1907/2006 (the REACH Regulation). This information has not been reviewed or verified by the Agency or any other authority. The content is subject to change without prior notice. The information in the C&L Inventory … comes from the C&L notifications. This information has not been reviewed or verified by the Agency or any other authority. The content is subject to change without prior notice. The Agency cannot guarantee the correctness of the information in the databases and the REACH Regulation does not permit it to make modifications to the data provided by the owner(s) of the respective information.

[72] Existing Substance Regulation

Appendix V: The Threshold of Toxicological Concern

A health risk assessment is ideally carried out on the basis of substance-specific toxicity data but, where these (or suitable read-across data) are lacking, default values for tolerable exposures can be assigned by applying a Threshold of Toxicological Concern (TTC) approach. A risk assessment can then be carried out by comparing the estimated exposure with the default values for tolerable exposure. Currently, proposed TTC values are available for acute and repeated exposure to mutagens (or suspected mutagens) and non-mutagenic (threshold) toxins.

The TTC concept is based on the reasoning that the toxicological properties of a universe of tested chemicals can be used to make conservative predictions of the likely toxicity of a larger universe of untested chemicals. In particular, the aim is to define a low level of exposure of an untested chemical that is highly unlikely to pose any significant toxicological risk. This allows the possibility of a satisfactory and health-precautionary risk assessment to be undertaken in the absence of toxicity data on the compound itself. The TTC approach is the scientific foundation of the US FDA (US Food and Drug Administration) Threshold of Regulation for indirect food additives, of the evaluations of food flavouring substances both by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2009) and within the European Food Safety Authority (EFSA, 2009, 2012a), and of the guidelines of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and European Medicines Agency (EMA) that define acceptable limits of mutagenic impurities in pharmaceutical products (EMA, 2006, 2010, 2015; ICH, 2017).

The TTC principle refers to the establishment of generic human exposure threshold values for (groups of) chemicals below which there would be no appreciable risk to human health. TTC values have been proposed following the statistical analysis of the oral toxicity data on large numbers of tested chemicals. These analyses incorporated numerous study NOAELs (no-observed-adverse-effect levels) for non-carcinogens, while for genotoxic carcinogens the focus was on the lifetime daily doses estimated to be associated with “insignificant” increases in cancer risk (1 extra case in 1,000,000 people exposed daily for lifetime) (Cheeseman *et al.*, 1999; EFSA, 2012a; Felter *et al.*, 2009; Kroes *et al.*, 2000, 2004; Munro *et al.*, 1996, 1999; SCCS/SCHER/SCENIHR, 2012). The TTC concept is not applicable to certain groups of substances e.g. heavy metals and other inorganics, polyhalogenated dibenzo-p-dioxins, polyhalogenated dibenzofurans, polyhalogenated biphenyls, compounds that accumulate in the body, endocrine disrupting chemicals (including steroids), high molecular weight (MW) chemicals such as polymers (not defined but polymers are often described as having MWs >1000 Daltons), and proteins (Cheeseman *et al.*, 1999; Kroes *et al.*, 2004), substances predicted to have local effects on the gastro-intestinal tract, nanomaterials and substances with a high potential for bioaccumulation (EFSA, 2012a). Also excluded are substances that are structurally related to certain established carcinogens of unusually-high potency (the so-called cohort of concern e.g. N-nitroso, azoxy, aflatoxin, benzidine and hydrazine compounds) (Kroes *et al.*, 2004; SCCS/SCHER/SCENIHR, 2012). Recently, EFSA has proposed that hydrazines can be removed from this cohort on the basis that cancer risk from their lower potency is adequately controlled by the default TTC figures (EFSA, 2019).

One of the most critical, lifetime exposure TTC values is 0.15 µg/person/day for chemicals with structural alerts for genotoxicity (Kroes *et al.*, 2004). As regards the non-genotoxins, lifetime TTC values of 18 (for organophosphates [OPs] and carbamates only) or 90, 540 or 1800 µg/day have been proposed, the choice of the toxicity TTC generally being influenced by broad structural class [the so-called Cramer Class] (Cramer *et al.*, 1978; EFSA, 2019; Kroes *et al.*, 2004; Munro *et al.*, 1999). These all relate to oral exposure[73].

EFSA concluded, based on a joint EFSA/WHO meeting, that "The TTC approach as currently applied is a valid, science-based screening tool useful for the prioritisation of chemicals and for more general applications in chemical risk assessment" (EFSA/WHO, 2015, 2016). In a very recent draft updated report, EFSA stated that "The threshold of toxicological concern (TTC) approach is a pragmatic, scientifically valid methodology to assess the safety of substances of unknown toxicity found in food and the environment. From a scientific perspective, the TTC approach could, in principle, be applied to any substances with known structure and that do not belong to the chemical exclusion categories, for which oral exposures can be estimated and toxicity data are sparse." EFSA went on to advise that "in the case of mixtures that are not fully defined, the application of the TTC approach may be acceptable if sufficient information or analysis is available to confirm that the mixture does not contain substances from the exclusion categories. In this case, the unknown components could be treated as potentially genotoxic and the TTC value of 0.0025 µg/kg bw would apply to the sum of these (mixture) components. If it were determined that there are no concerns for genotoxicity and the mixture does not contain organophosphates or carbamates, the mixture may be placed directly in Cramer Class III" (EFSA, 2019).

ICH/EMA guidance states that a TTC value of 1.5 µg/day (corresponding to a 1 in 100,000 cancer risk) can be justified for unavoidable genotoxic impurities in pharmaceutical products intended for long-term use, because these products provide a health benefit. A TTC value higher than 1.5 µg/day may be acceptable under certain conditions e.g. short-term exposure, for treatment of a life-threatening condition, when life expectancy is less than 5 years, or where the impurity is a known substance and human exposure will be much greater from other sources (e.g. food) (EMA, 2006, 2010, 2015; ICH, 2017). Guidance from EMA and ICH also addresses acute and other less-than-lifetime (LTL) exposures to unavoidable mutagenic impurities in pharmaceuticals. This applies an approach in which the acceptable cumulative lifetime dose (1.5 µg/day x 25,550 days = 38.3 mg) is uniformly distributed over the total number of exposure days during LTL exposure. This would allow higher daily intake of mutagenic impurities than would be the case for lifetime exposure and still maintain comparable risk levels for daily and non-daily treatment regimens. In the case of intermittent (non-daily) dosing, the acceptable intake is capped by the total cumulative dose or the maximum acceptable intake (i.e. 120 µg/day), whichever is lower (EMA, 2015; ICH, 2017). Tables 1 and 2 below illustrate the default acceptable intakes for acute to lifetime exposures to individual and total mutagens in pharmaceuticals (for both clinical development and marketing).

[73] Strictly speaking the various TTC threshold values only apply to oral exposure because the TTC database that is their foundation comprises only oral studies. Nevertheless, ICH guidance has confirmed that, for mutagens, the values generally apply to any route of administration (ICH, 2017).

Table (V) 1. Acceptable intakes for individual mutagens by duration (EMA, 2015; ICH, 2017)

Duration of treatment	<1 month	>1-12 months	>1-10 years	>10 years-lifetime
Daily intake (µg/day)	120	20	10	1.5

Table (V) 2. Acceptable intakes for total mutagens by duration (EMA, 2015; ICH, 2017)

Duration of treatment	<1 month	>1-12 months	>1-10 years	>10 years-lifetime
Daily intake (µg/day)	120	60	30	5

An ISO technical specification document on the application of the TTC for assessing medical device constituents is in agreement with the thresholds derived in ICH M7 (Table 1, above), further stating that these values are protective for carcinogens, systemic toxicants and reproductive toxicants. The technical document also recommends the use of Cramer Class TTC figures when experimental data or appropriate model-derived predictions (as described in ICH M7) suggest that a substance is unlikely to have carcinogenic effects (ISO, 2019).

Official guidance for acute TTC values applicable to non-mutagens is more limited. However, the EFSA Panel on Plant Protection Products and their Residues has recently proposed that adoption of a general oral TTC value of 5 µg/kg bw (300 µg for a person weighing 60 kg) could assist in the toxicological evaluation of pesticide metabolites and degradants. A lower TTC of 0.3 µg/kg bw (18 µg for a 60-kg person) was proposed for compounds containing an alert for neurotoxicity. These values were derived from a database of short-term NOAELs from studies that had been used in the derivation of Acute Reference Doses (ARfDs) for 217 non-neurotoxic and 41 neurotoxic pesticides. The TTC values were derived by applying a standard default Uncertainty Factor of 100 to the 5th percentile values of the two sets of NOAELs. The 5 µg/kg bw value was stated to be applicable to (non-neurotoxic) Cramer Class II and III compounds [there were no Cramer Class I compounds in the analysed data set] (EFSA, 2012b). Although these values were developed primarily for use in the pesticide sector, extension to chemicals more generally would seem to be health precautionary, given that pesticides by nature are designed to possess some toxic character.

A publication by a working group of the Product Quality Research Institute[74] (PQRI) has provided further guidance on assessment of extractables and leachables in parenteral and ophthalmic drug products (PODPs). The paper suggested a safety concern threshold (SCT) of 1.5 µg/day for genotoxic extractables and leachables in PODPs, and Qualification Thresholds (QTs) were initially set at 5 µg/day for sensitisers (and irritants) and 150 µg/day for all other compounds (showing systemic toxicity). The SCT was defined as a threshold below which a leachable would have a dose so low as to present negligible safety concern from carcinogenic and non-carcinogenic effects. This SCT was said to differ from a TTC because it is used primarily as a benchmark for identification purposes, rather than as a safety control limit (Paskiet *et al.*, 2013). PQRI scientists later proposed reducing the PODP QT figure for

[74] The Product Quality Research Institute (PQRI) is "a non-profit consortium of organizations working together to generate and share timely, relevant, and impactful information that advances drug product quality and development." Member organisations are the Consumer Healthcare Products Association, the U.S. Food and Drug Administration Center for Drug Evaluation and Research, Health Canada, the Americas International Pharmaceutical Excipients Council of the Americas, the International Society for Pharmaceutical Engineering, the Parenteral Drug Association and the United States Pharmacopeia.

substances showing only systemic toxicity from 150 to 50 µg/day (Paskiet, 2016). In the light of US FDA concern that 5 µg/day might not be appropriate for ophthalmics (where local irritancy might be more critical for these low-volume instillations), PQRI has proposed that the SCT (1.5 µg/day for mutagens) and QTs (5 and 50 µg/day for sensitisers and other general systemic threshold toxins, respectively) should apply only to parenteral drug products (PDPs). The SCT of 1.5 µg/day still applies for ophthalmics but no QTs are proposed for these products (McGovern, 2018).

For orally-inhaled and nasal drug products (OINDPs), PQRI originally proposed 0.15 µg/day as an SCT (for mutagens) and 5 µg/day as a QT (for non-mutagens) (Norwood *et al.*, 2013). More recently, in the light of ICH M7, US FDA has observed that 0.15 µg/day should continue to serve as an identification threshold for OINDPs, but that health (cancer) risk assessment will generally be carried out against the M7 1.5 µg/day benchmark for mutagens (McGovern, 2018).

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