TECHNICAL DOSSIER

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LEADING E-LIQUID
QUALITY

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TD0066

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This Technical Dossier has been generated by EL-Science for the customer detailed below. The Technical Dossier provides all analytical test results and toxicological data relevant to the specified flavours/ECID numbers.

SUBMITTER DETAILS

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MANUFACTURER DETAILS

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Country of Manufacture	United Kingdom (GB)

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ABOUT EL-SCIENCE

EL-Science are providers of technical solutions to the electronic cigarette industry.

Dedicated solely to eLiquid analysis and manufacture, EL-Science is one of the most experienced e-cigarette testing and manufacturing laboratories in Europe. With a leading team of scientists comprised of analytical, flavour, organic and toxicology chemists, EL-Science works closely in conjunction with their toxicological consultants, Bibra Toxicology Advise and Consulting.

EL-Science strongly feels that the quality of eLiquid across the UK can be enhanced through controlled production processes, tested and regulated vaping products, and good scientific research.

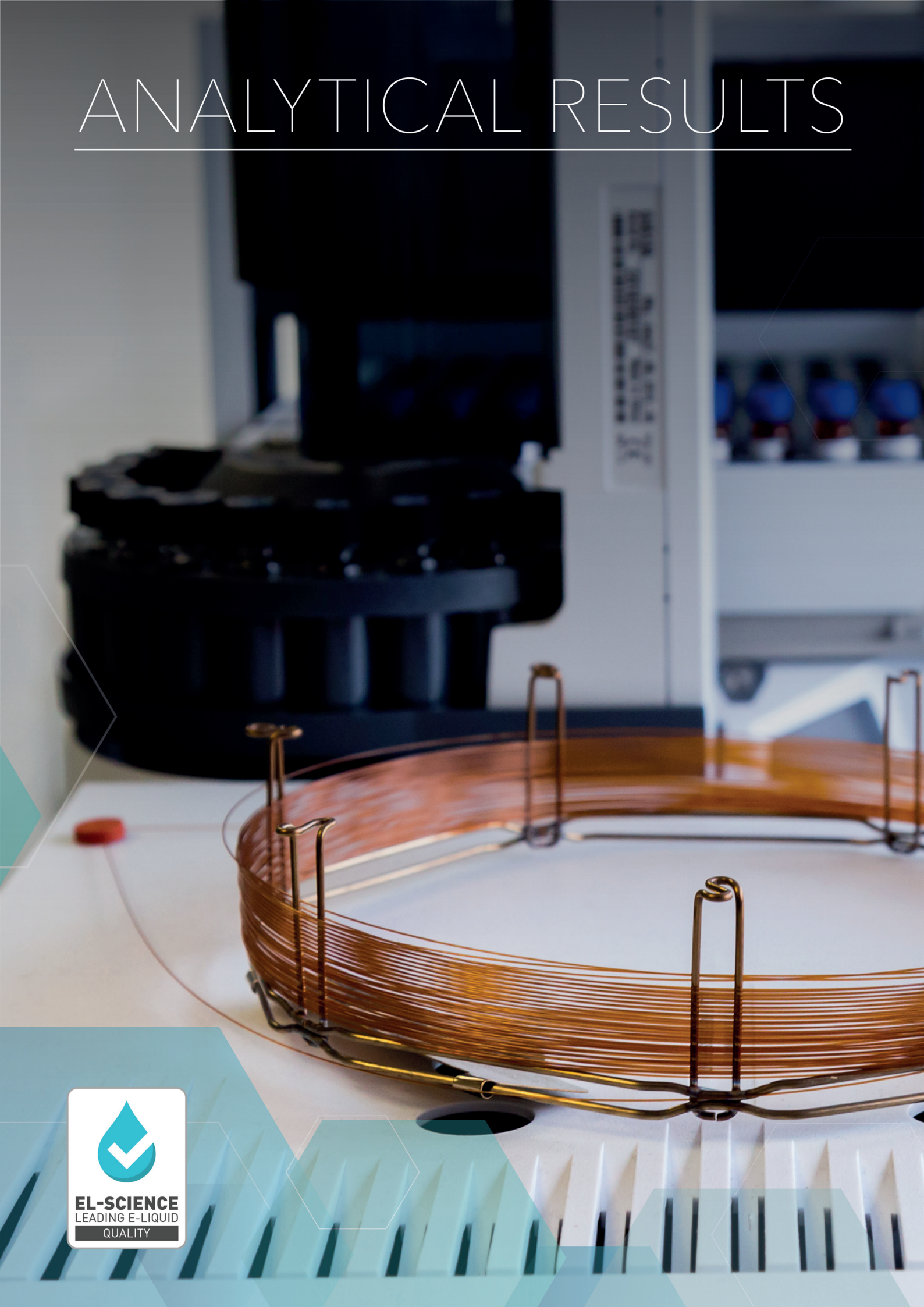
The Analytical Laboratories are specifically equipped to provide a full analytical service to ensure that tested eLiquids are compliant with Article 20 of the Tobacco Products Directive (TPD) (2014/40/EU), which came into effect in May 2016. Multiple methods of chemical analysis are applied to identify the compositional components of each eLiquid product, including gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography (HPLC) and inductively coupled plasma-mass spectrometry (ICP-MS). This allows for both the qualification and quantification of hundreds of flavour ingredients, as well as potentially harmful additives and undesirable contaminants. Emissions testing is performed using in-house developed electronic cigarette vapour collection device and using hardware, power settings and puff profiles that are relevant to and representative of the product being tested and that realistically simulate an end product user.

Once the product ingredients have been identified by the Analytical Team and the concentrations of each determined, they are risk assessed toxicologically by the Flavour Team, who not only consider the health implications to the end user of each individual ingredient present, but examine the additive effects of all the ingredients present together to assess the full eLiquid product as a whole. EL-Science and Bibra have determined Health Criteria Values (HCVs) for all chemicals which have been identified as having the potential to be detrimental to human health. These have been established by reviewing evidence from occupational and epidemiological studies, laboratory animal studies, and also, where available, from an understanding of the chemical's ADME (adsorption, distribution, metabolism, and excretion) and mechanism/mode-of-action. While assessment of HCVs is above and beyond the requirements of the TPD, it is an essential requirement of the General Product Safety Regulations 2005.

If a product does not meet the toxicological requirements, EL-Science can reformulate it for the Customer. The focus is on maintaining the flavour profile of the eLiquid as much as possible to maintain its originality, ensuring the integrity of the product's sensory profile, whilst reducing or entirely eliminating the compounds of toxicological concern.

For further information on EL-Science's TPD services or the other services that are offered, including manufacturing, white label, batch testing, and wholesale, please visit www.elscience.co.uk or email contact@elscience.co.uk.

ANALYTICAL RESULTS



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ANALYTICAL RESULTS

The following section lays out the details and data for the named product(s), in fulfilment of the producer's obligations under the Tobacco Products Directive (TPD) (2014/40/EU). The report details the quantities of flavourings/compounds identified in the starting eLiquid, and the levels of the mandatory compounds listed for emissions analysis.

2.1 GC-MS Compositional Testing Method Statement

Broad scan GC-MS analysis was employed to assist with the compositional identification of the eLiquid product. This was followed by targeted full-scanning analysis and selected ion monitoring (SIM) analysis to quantify the components identified. Both external and internal standards, including isotopically labelled (deuterated) standards were used.

Analytical quality control was maintained by a number of measures, including:
- 5 point calibration per targeted analyte prior to any sample analysis.
- Analysis of reagent / method blanks and matrix spike samples within each analytical batch.

Care has been taken to exclude from the quantitation those components identified which are believed to be analytical artefacts, such as from GC column bleed, solvent impurities, solvent/flavourant interactions and so on, however, there remains a chance of some being included in reported results. Again, care has been taken to identify precise structural and stereo isomeric forms of the various flavourings, however, in some cases mixed isomers may be reported as a single identity.

2.1.1 Equipment

Instruments:
- Agilent 7890B Gas Chromatograph (GC) fitted with 7693A autosampler and interfaced to 5977B Inert Plus Turbo MSD Mass Spectrometer (MS), configured with MassHunter software.
- Perkin Elmer Clarus 680 Gas Chromatograph (GC) fitted with autosampler, interfaced to Clarus SQ 8T Mass Spectrometer (MS), configured with Turbomass software.

Principal Column for quantitative analysis:
- 60 m x 0.25 mm x 0.25 µm DBWax (Agilent).

2.1.2 Limits of Detection (LOD) and Limits of Quantitation (LOQ)

Due to the large number of compounds being screened (400+) and the variability of the matrix between samples, the LOQ for all compounds was set at 50 ppm, except for diacetyl and acetylpropionyl which had 1 ppm LOQs. However, calculated detection limits were significantly lower, and the table below illustrates a selection of these calculated for components of representative functionalities.

2.1.4 Linear Retention Indices (LRIs)

To assist mass spectrometric identification of the analytes, linear retention indices (LRIs) were measured for the majority of the compounds on DBWax, DB1 and DB5 GC columns. Where standards were not available, literature values were used where possible to assist with the identification. The LRIs were calculated using a standard alkane mix from C7 to C30 run under identical GC conditions to the samples and authentic standards.

$$\mathbf{LRI_x = 100\left(\frac{RT_x - RT_{n0}}{RT_{n1} - RT_{n0}} + n0\right)}$$

Where:

x	=	target compound
n0	=	n-alkane eluting directly before x
n1	=	n-alkane eluting directly after x
RT	=	retention time (min)
LRI	=	linear retention index

2.3 Emissions Testing Method Statement

Eliquid emissions were tested as per intended hardware use. PG heavy samples were tested using disposable 1.6 mL clearomizers (Innokin Technology) using 2.5 ohm coils. A fresh clearomizer was used for each sample. VG heavy eLiquid samples were tested using Aspire Atlantis EVO sub-ohm tanks (Aspire) with 0.4 ohm atomizers. A new atomizer was used for each sample and tanks were cleaned in between samples. Eliquid samples with a PG:VG ratio of 50:50 were tested using both types of hardware as they can be used in either. As the EU-CEG portal only allowed for one data set to be uploaded, and products with 50:50 PG:VG ratios are more likely to be used in lower power devices (second generation vs third generation), the data gathered using the 2.5 ohm coils and clearomizers was used for TPD submissions, however both data sets are shown here. Clearomizers and tanks were allowed to prime for an hour before emission testing to ensure the eLiquid fully and consistently soaked into the wick or atomizer.

2.3.1 Collection Equipment

Vaping was simulated automatically using a 10 A programmable power supply (ISO-TECH IPS2010 bench power supply, output: 0 to 20 V, 0 to 10 A, 200 W) and digital mass flow controller (EL-FLOW Select, 4-200 mL s-1, Bronkhorst). Both devices were connected to custom-built LabVIEW control software. The software enabled the collection of a set volume of eLiquid emission, with the analyst being able to control the vaping pattern, collection volume, the output power setting and the flow rate.

2.3.2 Puffing Regime

A total of 1.1 L of emission was generated from each eLiquid sample using the following puffing conditions:
- 55 mL puff volume
- 3 s puff duration
- 20 s interval between puffs
- 20 inhalations.

2.3.3 Power Setting

The power setting used was dependent upon the PG to VG ratio of the eLiquid sample being tested, and was relevant to the intended product use. PG heavy samples in clearomizers with 2.5 ohm coils were tested at the standard 3.7 V. VG heavy samples were tested in sub-ohm tanks with 0.4 ohm coils and a higher power setting of 40 W.

2.3.4 GC-MS Equipment

Instrument:
- Perkin Elmer Clarus 680 Gas Chromatograph (GC) fitted with autosampler, interfaced to Clarus SQ 8T Mass

Spectrometer (MS), configured with Turbomass software.

Principal Columns for quantitative analysis:
- 60 m x 0.25 mm x 0.25 µm DBWax (Agilent)
- 60 m x 0.25 mm x 0.5 µm DBWax (Agilent)

2.3.5 GC-MS Analysis

Collected emission samples were analysed via GC-MS. A thicker film column was used to improve sensitivity for the more aldehyde compounds that elute at the start of the analysis. Both external and internal standards, including isotopically labelled (deuterated) standards were used.

Analytical quality control was maintained by a number of measures, including:
- 6 point calibration per targeted analyte prior to any sample analysis.
- Analysis of reagent / method blanks and matrix spike samples within each analytical batch.

2.3.6 Linear Retention Indices (LRIs)

A primary linear retention index was generated using a C7 to C30 hydrocarbon retention index standard and a modified version of Kovat's retention index equation, which allows for temperature programming of the GC system, as follows:

$$\mathbf{LRI_x = 100\left(\frac{RT_x - RT_{n0}}{RT_{n1} - RT_{n0}} + n0\right)}$$

Where:

x	=	target compound
n0	=	n-alkane eluting directly before x
n1	=	n-alkane eluting directly after x
RT	=	retention time (min)
LRI	=	linear retention index

Using the known straight chain hydrocarbons from the retention index standard, retention index values were assigned based on the carbon number of the component. The values assigned to each of the components were then plotted against their respective retention times to produce a linear retention ladder.

2.3.7 Limits of Detection (LOD) and Limits of Quantitation (LOQ)

Method		PG Heavy Samples using 3.7 V Clearomizer Method		VG Heavy Samples using Sub-Ohm Tank Method	
Target Analyte	CAS Number	LOD (µg/55 mL puff)	LOQ (µg/55 mL puff)	LOD (µg/55 mL puff)	LOQ (µg/55 mL puff)
Ethylene Glycol	107-21-1	0.0079	0.0238	0.0458	0.1387
Diethylene Glycol	111-46-6	0.0007	0.0022	0.0020	0.0060
Formaldehyde	50-00-0	0.0009	0.0026	0.0006	0.0019
Acetaldehyde	75-07-0	0.0076	0.0230	0.0058	0.0175
Acrolein	107-02-8	0.0010	0.0030	0.0024	0.0073
Crotonaldehyde	4170-30-3	0.0009	0.0026	0.003	0.0010
2,3-Butanedione	431-03-8	0.0036	0.0108	0.0032	0.0096
Acetylpropionyl	600-14-6	0.0007	0.0020	0.0009	0.0026

2.3.8 Calculated Emissions

EL-Science have provided an additional set of emissions data based upon the relationship between eLiquid and eLiquid vapour. The chemical composition has been processed to determine a worst case valuation of the quantity of a given chemical ingredient in the resulting vapour when a given eLiquid is atomised under standard conditions.

This data set is intended to assist the regulating authority with any subsequent hazard assessment or investigative work into such areas. EL-Science have used such an approach within our toxicological risk assessment of flavours with advice from Bibra Toxicology and Consulting Ltd. As a part of this assessment process, EL-Science have commissioned over £600,000 worth of toxicological data on over 500 of the most common flavour chemicals. These toxicological monographs are focused upon the effects of inhalation over a prolonged period and require a standard model of use to be assumed in order for subsequent Heath Criteria Values (HCVs) to be assessed and determined. EL-Science have taken this model and applied it to the chemical ingredients found within the eLiquid to provide a base for toxicological assessment in the vapourised form. The base of this standard model is presented in this document along with the calculations used to transpose the chemical composition in the eLiquid into that of the aerosol. This is possible because the electronic cigarette device effectively atomises the eLiquid, i.e. creating a colloidal collection of micro-liquid particles suspended in air, rather than boiling it, i.e. converting to a gas through a phase change, thus there is no theoretical plate differential and therefore the chemical composition remains unchanged. It is noted that chemical reactions do occur which is beyond the scope of this data set. It is hoped that this information will prove useful to regulating authorities or other research organisations who assess these data sets.

2.3.9 Eliquid Standard Assumptive Model (ESAM)

A standard assumption made within the calculation involved splitting eLiquid vaping habits into two categories:

1. Use of second generation or “standard” devices, such as the EGO battery with a clearomiser. This category was assigned an average daily intake of eLiquid of 1.5 mL per day. Use is primarily of PG heavy eLiquids.

2. Use of higher powered devices, such as variable wattage hardware designed for large amounts of vapour. This category was assigned an average daily intake of eLiquid of 4 mL per day. Use is primarily of VG heavy eLiquids (defined as having >50% VG).

Where an eLiquid had a 1:1 PG:VG ratio, the liquid was assumed to be PG biased as the standard use of these liquids trends towards the lower power, second generation products resulting in a use closer to 1.5 mL per day and smaller puff sizes.
Further assumptions were that:

- A user would consume on average 300 puffs per day.
- All chemicals present in the eLiquid transfer to the vapour phase, i.e. 100% recovery. This allows the presentation of a “worst case” concentration of each chemical per puff.
- Puff volume was to be standardised at 55 mL to allow comparison of data.

The units of the presented calculated data is in µg/55 mL puff.

The values presented in the EL-Science Standard Assumptive Model are open to substitution to fit the criteria of other organisations or researchers, whilst still allowing data to be compared between studies with a simple correction factor.

Each value with a calculated emission was determined using ESAM with the criteria detailed in the section above. The corresponding calculation is presented here:

$$C_v = \frac{(1000)(C_L)(L_d)(P_r)}{(P_n)(P_A)}$$

Where:

C_L	=	Concentration of the chemical in the e-Liquid (mg/mL)
C_V	=	Concentration of the chemical in the aerosol vapour (µg/puff)
P_n	=	Average number of puffs per day*
P_r	=	Real World Puff Size (mL)
P_A	=	Assumptive Puff Size (mL)*
L_d	=	Liquid consumed on average per day (mL)

* Used by ESAM during toxicological assessment and analytical testing regimes.

Note: C_v is representative of µg/P_r puff.

2.3.10 Metals Analysis

Elemental metals analysis was performed on the liquid samples via inductively coupled plasma-mass spectroscopy (ICP-MS). Results were then calculated from the liquid to the aerosol phase. Permitted daily exposure (PDE) limits are as recommended by the ICH Harmonised Guideline, Guideline for Elemental Impurities Q3D. As there are no established inhalation PDE for aluminium and iron, National Institute for Occupational Safety and Health (NIOSH) established recommended exposure limits (REL) for each (5,000 µg/m3) were used to calculated acceptable daily inhalation limits.

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Toxicological Risk Assessment

EL-Science commissioned Bibra Toxicology Advice and Consulting to assess the toxicity of each ingredient in this eLiquid formulation.

Bibra was founded (as the British Industrial Biological Research Association) in 1961 to provide independent, high-quality research, information and advice on chemical toxicology to industry and governmental departments. Its risk assessors have been working together for many years (40 years in some instances) and have a record of objectivity and scientific excellence. Eight scientists in the current team 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 professional conduct. Bibra has worked on thousands of human health hazard and risk assessments for hundreds of clients in a range of sectors, including (in more recent years) a large number of companies from the electronic cigarette industry. As such, Bibra's toxicologists have an impressive portfolio of relevant experience in assessing the chemical hazards and risks of substances added to, leached into, and/or emitted from, ecigarettes and eLiquids.

Bibra have prepared full or preliminary toxicity monograph on hundreds of eLiquid ingredients for EL-Science, including each of the ingredients in this eLiquid formulation. This involved initially searching for existing toxicity data in a range of data sources, reviewing the literature, and (where the data were considered key) briefly summarising that information. Each monograph is intended to be used as part of the eLiquid producers' Tobacco Products Directive (2014/40/EU) notifications, having regard to the common format for the notification of electronic cigarettes and refill containers as described in Commission Implementing Decision (EU 2015/2183) of 23 December 2015.

These literature reviews include sections on Irritation, Sensitisation, Acute and Repeated dose toxicity, Genotoxicity, Carcinogenicity, Reproductive and Developmental toxicity (i.e. CMR properties), Cardiopulmonary effects, and Addictiveness. The monographs focus on the inhalation route of exposure, but exclude data on cigarette smoke. EL-Science also commissioned Bibra to determine tolerable threshold levels (described in the relevant monographs as Health Criteria Values; or HCVs) for the toxicologically worst-case representative substance(s) in each structural class, where the data allowed. Where necessary (and possible from the data available), thresholds for local and systemic toxicity (cancer and non-cancer) were considered, and the lowest (i.e. most health precautionary) proposed. Exiting HCVs (e.g. OELs, US EPA RfCs, ATSDR MRLs, EFSA/JECFA ADIs/TDIs, etc.) already determined by Expert Groups were considered, as were ADME considerations, etc. Preferentially, these HCVs were determined on the basis of suitable points-of-departure (e.g. N/LOAEL/Cs) from reliable repeated-dose inhalation studies (human or laboratory animal), with the application of appropriate safety/uncertainty/assessment factors (primarily using ECHA REACH guidance as a basis). Where no inhalation studies were available, the use of studies involving other exposure routes (e.g. oral data) were considered, and appropriate route-to-route extrapolation factors introduced where needed. These HCVs were then converted to tolerable levels in the eLiquid (for PG- and VG-based formulations) based on assumptions regarding daily intake provided by EL-Science. While additive/ synergistic/antagonistic effects were considered as much as possible within chemical functional groups, a more comprehensive health risk assessment would allow for the full assessment of possible mixture interactions, particularly for those ingredients potentially acting via the same mode/mechanism of action. For further information on the commissioning of such an assessment, please email contact@elscience.co.uk or call +44 (0) 1733 352 553.

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Results

Flavour Name	Tiryaki
EL-Science Sample ID	RCP0504
Associated ECID Numbers	

INGREDIENTS LIST

ACTIVE INGREDIENT			
NAME	**CAS**	**REACH REG.**	**CONCENTRATIONS**
Nicotine	54-11-5	01-2120066934-47-xxxx	18 mg/mL

BASE INGREDIENTS			
NAME	**CAS**	**REACH REG.**	**RATIO (%)**
Propylene Glycol	57-55-6	01-2119456809-23-xxxx	25
Glycerine (VG)	56-81-5	01-2119471987-18-xxxx	75

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EMISSIONS DATA

MEASURED EMISSIONS (VG)			
NAME	CAS	IUPAC	QTY (µg/55mL puff)
Acrolein	107-02-8	Prop-2-enal	0.47292219
Formaldehyde	50-00-0	Formaldehyde	0.38744267
Acetaldehyde	75-07-0	acetaldehyde	0.15701707
Ethylene glycol	107-21-1	Ethane-1,2-diol	0.15433855
Diethylene glycol	111-46-6	2-(2-Hydroxyethoxy)ethan-1-ol	0.06126016
Acetylpropionyl	600-14-6	2,3-Pentanedione	Not Detected
Diacetyl	431-03-8	butane-2,3-dione	Not Detected
Crotonaldehyde	4170-30-3	(2E)-but-2-enal	Not Detected

MEASURED METAL			
NAME	CAS	INHALATION PDE (µg/day)	QTY (µg/day)
Aluminium (Al)	7429-90-5	82.5	<82.5
Chromium (Cr)	7440-47-3	2.9	<2.9
Iron (Fe)	7439-89-6	82.5	<82.5
Nickel (Ni)	7440-02-0	6.0	<6.0
Tin (Sn)	7440-31-5	64.0	<64.0
Lead (Pb)	7439-92-1	5.0	<5.0
Mercury (Hg)	7439-97-6	1.2	<1.2
Cadmium (Cd)	7440-43-9	1.7	<1.7

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NICOTINE DOSING (VG POWER SETTING)	
CONCENTRATION (mg/mL)	**DOSE (µg/55mL puff)**
18	240

RISK ASSESSMENT SUMMARY

USE IN THIRD GENERATION DEVICE (VG POWER SETTING)

EL-Science can confirm that the vast majority of individual ingredients in this eLiquid are present at a concentration leading to exposures that are lower than the appropriate determined tolerable levels, based on the HCV approach outlined above. One or more ingredients are at a slightly higher concentration that may result in some mild and short-lived adverse effects with heavy, long term use. These are indicated in the Ingredients List by the presence of an asterix (*). Reformulation is not required but should be considered.

TOXICOLOGICAL DATA

For more information, contact EL-Science

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