TÜV NORD Umweltschutz

Sector: Hazardous substances

Umweltschutz

SUMMARISED REPORT

on the analysis of a test liquid for the presence of harmful substances

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Analytical laboratory accredited by DAkkS -Deutsche Akkreditierungsstelle GmbH in accordance with DIN EN ISO/IEC 17025:2005 (Registration No. D-PL-14334-01-00).

The accreditation applies to the analytical methods cited in this report.

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1. Assignment

Herr Ralf Steffan of Happy People UG commissioned TÜV NORD Umweltschutz GmbH & Co. KG to analyse the test liquid 'Happy-Liquid Menthol High 18 mg/ml Nicotin'. According to the client, this is an e-liquid used as a filling for electronic cigarettes. The e-liquid itself is a blend of various substances whereby the vapour is generated by its main constituent. Also present in the e-liquid are flavourings and nicotine.

We were commissioned to determine the levels of the following substances in the test liquid:

- Formaldehyde
- VOCs
- Amines
- Nitrosamines

Levels were to be determined in the untreated test liquid and in the gas phase. For this purpose, the test liquid was to be heated to 80°C – as in the case of the e-cigarette that was placed at our disposal – and the constituents of the e-liquid that were then present in air were to be analysed.

2. Analytical methodology

2.1 Analysis in a test container

The test liquid was transferred to a glass container that was then heated to 80°C. Relative to their individual boiling point, the organic compounds present in the test liquid entered the gas phase and, following a saturation of phase of 30 minutes, samples of this were then taken, whereby the removed gas phase was replaced with nitrogen. The purpose was to simulate the composition of the gases generated during the smoking of the e-cigarette.

The air from the test container was analysed as described in the following.

2.2 Analysis of volatile organochemical substances in air samples using thermal desorption and GC/MS (per work sheet AB283059)

Samples for analysis were prepared in accordance with VDI guideline 2100 sheet 3. This guideline specifies that samples of air are to be drawn through an adsorption tube packed with Tenax. Organic substances present in the air are absorbed and accumulate in the Tenax adsorbent. Following supplementation with an internal standard in the laboratory, desorption is undertaken and the samples analysed by means of capillary gas chromatography/mass spectroscopy. This analytical technique makes it possible to separate very complex mixtures of substances.
Following analysis, possible identifications of the individual substances were obtained by means of an automatic search of a Wiley database for matches with the mass spectra obtained. Final identifications were undertaken with the aid of the TÜV Nord ambient air database. Retention indices were calculated for the detected substances and the supplementary internal standard (similar to those of the Kovats retention index). The potential identifications of the mass spectra generated with the aid of the database and the retention indices were tested for plausibility and the final identification of substances was based on the results.

A semi-quantitative estimate of concentrations of each substance (as toluene equivalents) was based on the corresponding AUCs.

The error level of this method is substance-dependent; in the case of selected standard compounds (aromatics) it is approx. 30%. The detection limit is, relative to toluene, approx. 1 µg/m^3.

2.3 Analysis of formaldehyde levels in air samples; VDI 3862 sheet 3 dated 09/99

Samples were acquired by means of adsorption of the formaldehyde present in the sample medium using silica gel coated with 2,4-dinitrophenylhydrazine (DNPH). Formaldehyde reacts with the DNHP coating of the silica gel to form the corresponding hydrazone. In a departure from the VDI method, this hydrazone was removed from the silica gel using DNPH solution (c = 0.1 mg/ml).
HPLC (high performance liquid chromatography) and the external standard method were used for the quantitative analysis of the formaldehyde with UV detection at 360 nm. Method error is ≤10%.

2.4. Analysis of nitrosamines

The air to be analysed was drawn through a Thermosorb/N adsorption tube resulting in the defined accumulation of the N-nitrosames present.

The sampling and analysis were performed in accordance with the standard BGI 505-23 method (issue 09/1992 method No. 4).

The principle is based on dynamic elution of the adsorbed N-nitrosamines using a mixture of dichloromethane/methanol and subsequent gas chromatographic analysis using a thermal energy analyser (TEA) detector. The sample was analysed for the presence of the following highly volatile N-nitrosamines that are classified as carcinogens:

- N-nitrosodimethylamine
- N-nitrosomethylethylamine
- N-nitrosodiethylamine
- N-nitrosodi-i-propylamine
- N-nitrosodi-n-propylamine
- N-nitrosodi-n-butylamine
- N-nitrosopiperidine
- N-nitrosopyrrolidine
- N-nitrosomorpholine

Analysis was undertaken in our accredited partner laboratory, Deutsches Institut für Kautschuktechnologie e.V. in Hanover.

2.5 Analysis of primary and secondary amines, TÜV work sheet 283051

Sampling was performed by means of adsorption of the amines present in the sample medium by surface-modified silica gel. The amines were then eluted in a desorption apparatus using alkaline methanol (2% KOH in methanol). Added to an aliquot of the sample solution was 9-fluorenylmethyl chloroformate (FMOC-Cl). The amines reacted with this to form the corresponding stable carbamates. Quantitative analysis of amines was performed using HPLC and the external standard method with UV detection at $263_{ex}/323_{em}$ nm. The estimated method error is <30%.

3. Sampling and results

Sampling was undertaken by Dipl.-Ing. Alexander Höhn of TÜV NORD Umweltschutz GmbH & Co. KG on 4 December 2013.

3.1 Analysis of volatile organochemical substances in air samples using thermal desorption and GC/MS

Fig. 1 in the following shows the total ion current chromatogram (TIC). Listed below the TIC are the largest AUCs with their standardised retention times (RT), the identification nos. of the Chemical Abstracts Service (CAS), the possible identifications and concentrations (ng/sample) in toluene equivalents.

Notes on the identification table:

- Specific substance name (e.g. toluene): the **retention index** of a **reference substance** analysed with the system and its **mass spectrum** are identical with those of the detected substance.

- Specific substance name prefixed by '?' (e.g. ? 1-butoxy-2-propanol): the **retention index** quoted in the **literature** (where given) **and** the **mass spectrum** in the online **WILEY database** or the TÜV Nord **ambient air database** are identical with those of the detected substance.

- Compound name prefixed by '?' (e.g. ? acetic acid ester): the **mass spectrum** in the online **WILEY database** or the TÜV Nord **ambient air database** is **similar** to that of the detected substance: characteristic moieties indicate it is this compound; there is **no** additional information on the **retention index.**

- Substance class (e.g. C-4 aromatic): the **mass spectrum** in the online **WILEY database** or the TÜV Nord **ambient air database** is **identical** with that of the detected substance; there is **no** additional information on the **retention index.**

- Substance class prefixed by '?' (e.g. ? C-12 alkane): the **mass spectrum** in the online **WILEY database** or the TÜV Nord **ambient air database** is **similar** to that of the detected substance; characteristic moieties indicate that it is of this substance class; there is **no** additional information on the **retention index.**

Analysis of the liquid by means of GC-MS screening

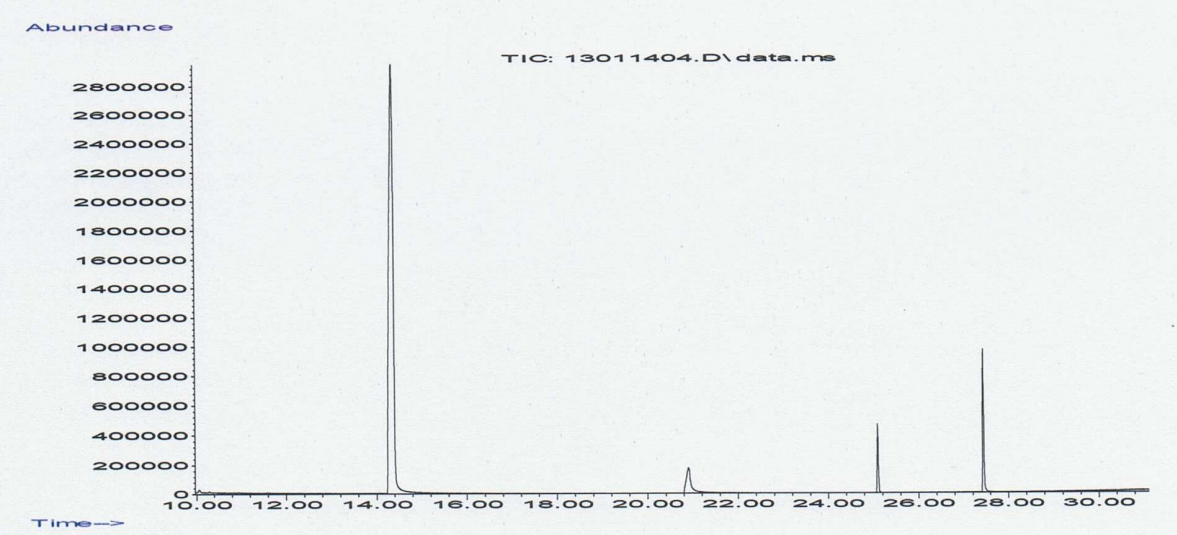

Table: identified substances

Ret. time	AUC%	Substance
14.378	83.1	1.2-propanediol (propylene glycol)
20.902	6.0	Glycerol
25.094	3.5	Menthol
27.445	7.5	Nicotine

3.2 Analysis of volatile organochemical substances in the gas phase

A fraction of the sample was analysed using headspace GC-MS. For this purpose, the liquid was heated to 80°C for several hours. An aliquot was removed from the gas phase using a gastight syringe and injected into the gas chromatograph. The mass selective detector was set to scan mode to detect the separated substances. The detected substances were identified with the help of the Wiley 275/NIST 05 spectrum library supplied with the device.

Analysis of the gas phase by means of headspace GC-MS

Chromatogram

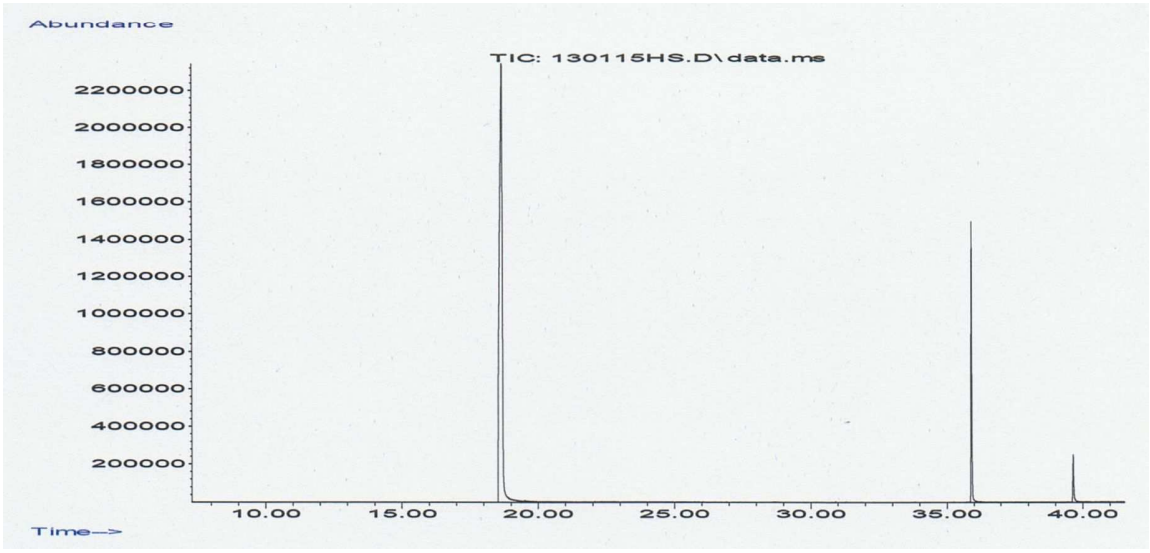

Table: identified substances

Ret. time	AUC%	Substance
18.673	80.4	1.2-propanediol (propylene glycol)
35.897	16.5	Menthol
39.646	3.1	Nicotine

3.3 Analysis of levels of formaldehyde in the gas phase and the test liquid

The concentration of formaldehyde in the gas phase at a temperature of 80°C was 20.7 µg/m^3.
The concentration of formaldehyde in the test liquid was 0.005 µg/mg.

Note: The currently recommended upper threshold for the concentration of formaldehyde in enclosed spaces is 120 µg/m^3.

3.4 Analysis of nitrosamines

The concentrations of all N-nitrosamines were below the detection limit in both the test liquid and the gas phase.

The absolute detection limits for the individual N-nitrosamines are in the range 1.4 pg - 4.2 pg.

Hence, concentrations were below 0.2 µg/m^3 in the gas phase and below 0.07 µg/ml in the test liquid.

Samples were analysed for the presence of the following nitrosamines:
N-nitrosodimethylamine (NDMA)
N-nitrosomethylethylamine (NMEA)
N-nitrosodiethylamine (NDEA)
N-nitrosodiisopropylamine (NDiPA)
N-nitrosodipropylamine (NDPA)
N-nitrososdibutylamine (NDBA)
N-nitrosopiperidine (NPIP)
N-nitrosopyrrolidine (NPYR)
N-nitrosomorpholine (NMOR)

3.5 Analysis of primary and secondary amines

Amines	In the gas phase µg/m^3	Test liquid µg/ml
Methylamine	87.03	2
Di-n-butylamine	24.36	4

Levels of other amines were below the detection limit.

The limits of quantification of the method when a standardised sample volume of 0.1 m^3 is used are:

	Concentration [µg/m^3]
Methylamine	**0.10**
Morpholine	**0.08**
Ethylamine	**0.13**
Dimethylamine	**0.06**
Pyrrolidine	**0.11**
2-butylamine	**0.13**
i-butylamine	**0.09**
1-butylamine	**0.08**
t-butylamine	**4.66**
Diethylamine	**0.10**
Aniline	**0.42**
Piperidine	**0.18**
N-methylaniline	**0.13**
Cyclohexylamine	**0.23**
1,2-diaminoethane	**0.04**
N-ethylaniline	**0.43**
Dipropylamine	**0.08**
Piperazine	**0.05**
Di-i-butylamine	**0.22**
Di-n-butylamine	**0.06**

4. Summary

Herr Ralf Steffan of Happy People UG commissioned TÜV NORD Umweltschutz GmbH & Co. KG to analyse the test liquid 'Happy-Liquid Menthol High 18 mg/ml Nicotin'. According to the client, this is an e-liquid used as a filling for electronic cigarettes.

The results of analysis of the untreated test liquid and its gas phase at 80°C can be found in section 3. We have not been commissioned to evaluate these results; should this be necessary, we recommend that this task is assigned to a toxicologist.

Dipl.-Ing. Alexander Höhn Dipl.-Ing. Wilfried Schwampe

Expert consultants of

TÜV NORD Umweltschutz GmbH & Co. KG