CHELAB S.R.L.

RAPPORTO DI PROVA 16/000496390
TEST REPORT N. 16/000496390

data di emissione /date of issue 23/12/2016

Codice intestatario
Customer ID 0076995

Spett.le/Messrs
FEM2 AMBIENTE SRL
PIAZZA DELLA SCIENZA, 2
20126 MILANO (MI)
IT

Dati campione
Sample information

Numero di accettazione
Acceptance number 16.410599.0012

Consegnato da
Delivered by GLS General Logistics Systems il 02/11/2016 / GLS General Logistics Systems on 02/11/2016

Data ricevimento
Receiving Date 02/11/2016

Proveniente da
Place of origin FEM2 AMBIENTE SRL PIAZZA DELLA SCIENZA, 2 20126 MILANO (MI) IT

Descrizione campione
Sample Description 00213-16-00054:PACIFIC SMOKE:TWELVE MONKEYS:HAMBOREE 12:00213-16-00054

Dati campionamento
Sampling information

Campionato da
Sampled by Cliente / Customer

Chelab S.r.l, a Mérieux NutriSciences company
Head office: Via Fratta 25 31023 Resana, Italy Phone. + 39 0423.7177 / Fax + 39 0423.715058 www.merieuxnutrisciences.it
VAT nr. 01500900269, R.E.A Treviso n. 156079 Fully paid up € 103.480,00.

RISULTATI ANALITICI
ANALYTICAL RESULTS

	Valore Value	U.M. Unit of measure	LoQ LoQ	LoD LoD	Data inizio fine analisi Start/end date of analysis	Unità op. Op. units	Riga Row
SUL CAMPIONE TAL QUALE / ON SAMPLE AS IT IS							1
NICOTINA / NICOTINE Met.: MP 2119 rev 1 2016	0,846	g/100 g	0,010	0,0050	04/11/2016- -21/11/2016	10	2
PREPARAZIONE DEL VAPORE EROGATO / PROCEDURE FOR PREPARATION RELEAESED STEAM Met.: AR 2016/284/B-CAP.2					04/11/2016- -21/11/2016	10	3
Numero di svapate / Puffs' number	100						4
Durata singola svapata / Puff time	3,0	s					5
Pausa tra due svapate / Lapse time	30	s					6
Dispositivo / Device	Nautilus X 15W / Nautilus X 15W						7
PREPARAZIONE DEL VAPORE EROGATO / PROCEDURE FOR PREPARATION RELEAESED STEAM Met.: AR 2016/284/B-CAP.1					04/11/2016- -21/11/2016	10	8
Numero di svapate / Puffs' number	100						9
Durata singola svapata / Puff time	3,0	s					10
Pausa tra due svapate / Lapse time	30	s					11
Dispositivo / Device	Nautilus X 15W / Nautilus X 15W						12
LIQUIDO PER SIGARETTE ELETTRONICHE - SUL VAPORE EROGATO / E-LIQUID FOR ELECTRONIC CIGARETTES - ON RELEASED STEAM							13
GLICOLI / GLYCOLS Met.: AR 2016/284/B-CAP.1 + AR 2010/046/A-CAP.6					04/11/2016- -05/12/2016	10	14
Glicole etilenico / Ethylene glycol	n.r. / n.d.	µg	100	50			15
Glicole dietilenico / Diethyleneglycol	n.r. / n.d.	µg	100	50			16
N-NITROSOAMMINE IN TABACCO / n-NITROSAMINE ON TOBACCO Met.: AR 2016/284/B-CAP.1 + AR 2012/009/A-CAP.6					02/11/2016- -05/12/2016	01	17
N'-nitrosonornicotina (NNN) / n'-Nitrosonornicotine (NNN)	n.r. / n.d.	µg	0,15	0,050			18
4-(n-metil-n-nitrosoammino)-1-(3-piridil)-1-butanone (NNK) / 4-(n-Methyl-n-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	n.r. / n.d.	µg	0,15	0,050			19
ARSENICO / ARSENIC Met.: AR 2016/284/B-CAP.1 + EPA 6020 B 2014	< LoQ / < LoQ	µg	0,050		02/11/2016- -29/11/2016	02	20
CADMIO / CADMIUM Met.: AR 2016/284/B-CAP.1 + EPA 6020 B 2014	< LoQ / < LoQ	µg	0,050		02/11/2016- -29/11/2016	02	21
CROMO / CHROMIUM Met.: AR 2016/284/B-CAP.1 + EPA 6020 B 2014	0,104	µg	0,050		02/11/2016- -29/11/2016	02	22
NICHEL / NICKEL Met.: AR 2016/284/B-CAP.1 + EPA 6020 B 2014	0,44	µg	0,050		02/11/2016- -29/11/2016	02	23
PIOMBO / LEAD Met.: AR 2016/284/B-CAP.1 + EPA 6020 B 2014	< LoQ / < LoQ	µg	0,050		02/11/2016- -29/11/2016	02	24
RAME / COPPER Met.: AR 2016/284/B-CAP.1 + EPA 6020 B 2014	0,058	µg	0,050		02/11/2016- -29/11/2016	02	25
NICOTINA / NICOTINE Met.: AR 2016/284/B-CAP.1 + MP 2119 rev 1 2016	6 970	µg	200		04/11/2016- -25/11/2016	10	26
COMPOSTI ORGANICI VOLATILI / VOLATILE ORGANIC COMPOUNDS Met.: AR 2016/284/B-CAP.1 + MP 2205 rev 0 2016					02/11/2016- -09/12/2016	02	27
Toluene / Toluene	< LoQ / < LoQ	µg	3,0				28
Benzene / Benzene	< LoQ / < LoQ	µg	3,0				29
1,3-butadiene / 1,3-Butadiene	< LoQ / < LoQ	µg	3,0				30
Isoprene (metil-butadiene) / Isoprene (methyl-butadiene)	< LoQ / < LoQ	µg	3,0				31
Diacetile / Diacetyl	< LoQ / < LoQ	µg	11				32
2,3-pentadione / 2,3-Pentanedione	< LoQ / < LoQ	µg	11				33

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RISULTATI ANALITICI
ANALYTICAL RESULTS

	Valore Value	U.M. Unit of measure	LoQ LoQ	LoD LoD	Data inizio fine analisi Start/end date of analysis	Unità op. Op. units	Riga Row
ALDEIDI / ALDEHYDES Met.: AR 2016/284/B-CAP.1 + MP 2204 rev 0 2016					02/11/2016- -05/12/2016	02	34
Acroleina / Acroleine	9,2	µg	5,1				35
Aldeide acetica / acetaldehyde	15	µg	4,2				36
Crotonaldeide / Crotonaldehyde	< LoQ / < LoQ	µg	6,0				37
Formaldeide / formaldehyde	72	µg	3,1				38
Propionaldeide / Propinaldehyde	< LoQ / < LoQ	µg	5,2				39
Butirraldeide e isobutirraldeide / Butyerraldehyde and isobutyrraldehyde	< LoQ / < LoQ	µg	12				40
TEST DI CITOTOSSICITA' IN VITRO / IN VITRO CITOTOXICITY TEST Met.: AR 2016/284/B-CAP.2 + SOPa 140 REV 3	vedi allegato / view attached file				02/11/2016- -22/12/2016	09	41

Informazioni aggiuntive /Supplement information

Riga \Row (20-25) - Metodo/Method: AR 2016/284/B-CAP.1 + EPA 6020 B 2014 = For the analysis performed by the EPA 6020 method, the LCS recovery (laboratory control sample) ranges between 85% and 115% as expected by the method.

Unità Operative /Operative units

Unità \Unit 10 : RESANA (VIA FRATTA - NON FOOD)
Unità \Unit 01 : Via Fratta Resana (TV)
Unità \Unit 02 : Via Castellana Resana (TV)
Unità \Unit 09 : Via Fratta Resana PHARMA (TV)

Chemical responsible Operative units 02,09	Chemical responsible Operative units 10	Laboratory manager
Dott. Federico Perin Chimico Ordine dei chimici - Provincia di treviso Iscrizione n. A338	Dott. Enrico Nieddu Chimico Ordine dei chimici - Provincia di treviso Iscrizione n. A339	Dott. Sébastien Moulard
Num. certificato 14114242 emesso dall'ente certificatore ArubaPEC S.p.A. NG CA 3, ArubaPEC S.p.A., IT	Num. certificato 14114239 emesso dall'ente certificatore ArubaPEC S.p.A. NG CA 3, ArubaPEC S.p.A., IT	Num. certificato 14114487 emesso dall'ente certificatore ArubaPEC S.p.A. NG CA 3, ArubaPEC S.p.A., IT

- LoD: limite di rilevabilità, individua un intervallo di confidenza dello zero ad un livello di probabilità del 99%. - LoQ: limite di quantificazione; "n.r.": non rilevato, indica un valore inferiore a LoD; "tracce (x)": indica un valore compreso tra LoD e LoQ, tale valore è puramente indicativo; "<x" o ">x" indicano rispettivamente un valore inferiore o superiore al campo di misura della prova. - Se non diversamente specificato, le sommatorie sono calcolate mediante il criterio del lower bound (L.B.). - Iscrizione al numero 7 dell'elenco regionale della Regione Veneto dei laboratori che effettuano analisi nell'ambito delle procedure di autocontrollo delle industrie alimentari, come da Allegato A del DDR n. 73 del 16 gennaio 2008.
- LoD is the detection limit and identifies a confidence interval of zero with a probabilty interval of about 99%. - LoQ is the limit of quantification."n.d" is not detected and indicates a value inferior to the LoD. "traces (X)" means a value between LoD and LoQ, this value is indicative. "<x" or ">x" indicate inferior or superior to the measurement field of the test. - If not differently specified, the sums are calculated by lower bound criteria (L.B.). - Registration with the number 7 of the Regional List of the laboratories of the Regione Veneto which perform analyses as regards the procedures for the food safety in food industries, as reported in Annex A of DDR n°73 of 16th January 2008

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RESULTS

Table 1:

	QUALITATIVE EVALUATION	OD*		RELATIVE VIABILITY (%)	
		Mean	SD	Mean	SD
Mean VC	0	0,316	0,020	100,0	6,3
Test sample	1	0,284	0,038	90,0	12,1
Positive control	3	0,101	0,023	32,0	7,4

*after blank subtraction

Acceptability criteria

Acceptability criteria were satisfied.

VIABILITY TEST SAMPLE: 90,0%

RESULT (viability ≥ 70% = non cytotoxic; viability < 70% = cytotoxic): NON CYTOTOXIC

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1. TEST SYSTEM

- Cell line: BALB 3T3 clone A31 - ATCC CCL-163 (Mammal fibroblasts)
- Culture conditions: 37 °C, 5% CO_2 atmosphere
- Growth medium: Dulbecco's Modified Eagle's Medium (D-MEM) with 10 % Newborn Calf Serum and 4 mM Glutammina
- Treatment medium: DMEM with 5 % Newborn Calf Serum and 4 mM Glutammina.

2. MATERIALS

- Purified Agar
- Dulbecco's Phosphate Buffered Saline (D-PBS) with Ca^{2+}/Mg^{2+}
- Dulbecco's Phosphate Buffered Saline (D-PBS) without Ca^{2+}/Mg^{2+}
- Neutral red (NR) solution 50 µg/ml: neutral red dye, medium DMEM
- Desorb solution: 49 % milliQ H_2O, 50 % ethanol, 1 % acetic acid
- Sodium dodecyl sulfate (SDS)

3. PREPARATION OF TESTING MATERIALS

- **Test item**: the sample was tested as it is
- **Positive control**: a 2 mg/ml SDS solution was prepared in D-PBS without Ca^{2+}/Mg^{2+}.
- **Negative control (Vehicle Control, VC):** treatment medium.

4. TREATMENT

- The day before the assay, cells were seeded in 18 wells of 3 6-well plates and incubated for 24 h at 37°C and 5%CO_2, allowing cell sedimentation and the constitution of a subconfluent monolayer.
- After 24 hours the 6 well plates were removed from the incubator and the growth medium was discarded.
- 2 ml of the 0.5 % Agar solution were added in each well. When the medium had solidified, 100 µl of the test sample were added on a sterile whatman filter (Ø approximately one tenth of the well) and the filter was applied on agar.
- Each sample (test item, positive control or vehicle control) was tested in 6 replicates.
- Plates were incubated for 24 h (37 °C; 5 % CO_2)
- After 24 hours prior to NRU assay, plates were observed to an inverted microscope and morphological anomalies caused by cytotoxicity were evaluated by a numerical index (see Tab. 2).

Tab. 2: Qualitative morphological grading of cytotoxicity

GRADE	REACTIVITY	CONDITIONS OF ALL CULTURES
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extending specimen size up to 1.0 cm
4	Severe	Zone extending farther than 1.0 cm beyond specimen

- The agar layer was removed and each well was rinsed with 2 ml of D-PBS with Ca^{2+}/Mg^{2+} and treated with 2 ml of 50 µg/ml NR solution for 3 h and incubated at 37 °C; 5 % CO_2.
- Afterwards each well was again rinsed with 2 ml of D-PBS with Ca^{2+}/Mg^{2+} and 1 ml of NR desorb solution were added.
- Plates were placed under gentle agitation in darkness for 10 min.
- Optical density of the NR extract at 540 nm was measured in a spectrophotometer.

5. DATA ELABORATION

- **Relative viability (RV)** represents the cell viability expressed as percentage with respect to the Vehicle Control (viability Vehicle control = 100 %).

(OD test item – OD blank) / (Media Vehicle Control – OD blank) x 100

6. ACCEPTABILITY CRITERIA

Quantitative evaluation

- Mean OD of VC > 0.2
- Mean relative viability for the positive control < 70 %

7. INTERPRETATION OF RESULTS

According to SOPa 140 rev.3, if the relative cell viability of the sample is < 70 % of the control group, then the material shall be considered cytotoxic, if ≥ 70 % of the control group, then the material shall be considered non-cytotoxic.

MERIEUX NutriSciences

Sinossi / Synopsis

Sigla metodo[1] / documento – *Method[1] / Document acronym*: MP-2119 rev.1

Titolo metodo / documento – *Method / document title*: DETERMINAZIONE DELLA NICOTINA IN LIQUIDO PER SIGARETTE ELETTRONICHE MEDIANTE HPLC/UV-VISIBILE / *DETERMINATION OF NICOTINE IN LIQUID FOR ELECTRONIC CIGARETTES BY HPLC/UV-VISIBLE*

Laboratorio / funzione – *Laboratory / Department*: Labdet-P

Data preparazione sinossi – *Synopsis preparation date*: 20/10/2016

Sinossi preparata da – *Synopsis prepared by*: L. Lovison

[1]come compare nei rapporti di prova / [1]*as appear in test report*

Sinossi / *Synopsis*

Il metodo descrive la procedura per la determinazione della nicotina in liquido per sigarette elettroniche. Il campione viene sciolto in opportuno solvente e analizzato in HPLC equipaggiato con rivelatore UV-visibile.

Sono state considerate le seguenti performance del metodo:

La conferma di identità si ottiene confrontando la scansione del picco del principio attivo nel cromato-gramma del campione con quella nel cromatogramma del materiale di riferimento. Gli andamenti delle scansioni devono risultare simili e i picchi massimi coincidere.

The method describes the procedure for determining nicotine in liquid for electronic cigarettes. The sample is dissolved in an appropriate solvent and analyzed by HPLC equipped with UV-visible detector.

The following performances of the method have been considered:
The confirmation of identity was obtained by comparing the scan of the peak of the active principle in the chromatogram of the sample with the one in the chromatogram of the reference material.
The trends of the scans should be similar and the highes peaks should coincide.

Sede legale e amministrativa: 31023 resana (tv) – via fratta, 25 tel. 0423.7177 (15 linee r.a.) – fax 0423.715058 – cosice fiscale, p. iva e reg. imprese tv 01500900269
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