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Safety Report on the Ruyan® e-cigarette Cartridge and Inhaled Aerosol

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Foreword

This safety report includes Health New Zealand's findings to date. Ruyan has allowed flexibility in the nature of investigations carried out. The tests reported are backed up by signed reports from the contracted laboratories. No completed test results have been withheld.

This version of the report includes results of further tests on mist and cartridges reported from the laboratories since the last version on 21 October 2008. The overall conclusions of the report are unchanged.

Source of funding and disclaimer

The Ruyan® e-cigarettes and the funds for testing them were supplied under a contract by Ruyan (Holdings) Ltd Hong Kong, but the findings are those of the author. Neither the author nor Health New Zealand Ltd holds stock in Ruyan (Holdings) Co. Ltd.

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30 October, 2008

Summary

Aim. This report aims to assist regulators in initial assessment of the safety of the Ruyan® e-cigarette and its cartridges, and the possible risks and benefits from permitting its use.

Method. Health New Zealand Ltd contracted with seven leading government, university and commercial laboratories in New Zealand and Canada to independently perform various tests on the Ruyan cigarette's nicotine refill cartridge.

Findings. Ruyan® e-cigarette is designed to be a safe alternative to smoking. The various test results confirm this is the case. It is very safe relative to cigarettes, and also safe in absolute terms on all measurements we have applied. Using micro-electronics it vaporizes, separately for each puff, very small quantities of *nicotine* dissolved in *propylene glycol*, two small well-known molecules with excellent safety profiles, – into a fine aerosol. Each puff contains one third to one half the nicotine in a tobacco cigarette's puff. The cartridge liquid is tobacco-free and no combustion occurs.

Competency. The author has authored or co-authored over 30 research papers and reports in national and international scientific medical journals since 1995, on smoking, and latterly on testing of cigarettes and cigarette substitutes.

www.healthnz.co.nz/Publicnsall.htm

Financial disclosure. This report is funded by Ruyan.

Disclaimer. Apart from research Health New Zealand derives no financial benefit from Ruyan.

Limitation. Unless the contrary is stated, findings refer specifically to Ruyan® e-cigarettes only.

Safety of the Ruyan® cartridge liquid and inhaled aerosol

1. Propylene Glycol

Summary: Propylene glycol (PG) is virtually non-toxic.

According to the manufacturer, propylene glycol makes up 89-90% of the liquid in the nicotine cartridge that generates the mist and vapor in the e-cigarette 'smoke'. (see Appendix 1.)

Properties and uses.

Propylene glycol $C_3H_8O_2$ is a completely water soluble liquid, and is prepared by hydrolysis of propylene oxide under pressure at high temperature without a catalyst. It is used in pharmaceuticals, as a drug vehicle (for example as an FDA approved solvent for intravenous diazepam) and preservative. It is used also in personal lubricants. It is used in semi-moist pet food and as a humectant for tobacco. In the food industry it is used as a solvent, humectant and preservative. Its mist is used in theatrical stage productions.¹ At low humidity, PG is a vapor; as dose and humidity increase it is held in the form of mist or fog, and at 100% humidity as in the lungs, it is dissolved.

Animal studies

Rats. In a study of rats exposed for 60 hours over two weeks, the highest concentration tested, 1800 mg/m³, which was the highest concentration that could practically be generated, was the no-observed-effect level (NOEL). PG does not appear to pose a significant hazard via inhalation of either the vapor or a vapor/aerosol mixture.²

Addition of propylene glycol at 2.2% w/w tobacco does not increase the toxicity of cigarette tobacco.³ In rats PG levels in plasma and lung are super-imposable within half an hour. A mild cumulative build up (30% or less) occurred after 28 days.⁴

¹ Office of Health and Environmental Assessment. EPA. Health and Environmental effects document for propylene glycol. ECAO-CIN-GO26. Prepared for Office of Solid Waste and emergency response. EPA 1987.

² Suber et al., Subchronic nose-only inhalation study of propylene glycol in Sprague-Dawley rats. Food Chem Toxicol 1989; 27:573-583.

³ Heck JD, Gaworski CL, Rajendrant N, et al. Toxicological evaluation of humectants added to cigarette tobacco: 13-week smoke inhalation study of glycerin and propylene glycol in Fischer 344 rats. Inhal Toxicol 2002;14: 1135-52.

⁴ Venitz J, Werley MS. Systemic and pulmonary pharmacokinetics (PK) of propylene glycol (PG) after inhalation of a condensation aerosol in rats for 28 days. Presented at AAPS annual meeting 2003, Salt Lake City.

http://www.chrysalis-technologies.com/publications/AAPS_Systemic%20and%20Pulmonary%20PK%20of%20PG.pdf

Monkeys, rats. Doses 50-700 times the amount absorbable from PG saturated air were administered for 12-18 months to monkeys. With a view to determining the safety of employing the vapors of propylene glycol and triethylene glycol in atmospheres inhabited by human beings, monkeys and rats were exposed continuously to high concentrations of these vapors for periods of 12 to 18 months. Equal numbers of control animals were maintained under physically similar conditions. The doses administered represented 50 to 700 times the amount of glycol the animal could absorb by breathing air saturated with the glycol.

Comparative observations on the growth rates, blood counts, urine examinations, kidney function tests, fertility and general condition of the test and control groups, exhibited no essential differences between them with the exception that the rats in the glycol atmospheres exhibited consistently higher weight gains.⁵

Propylene glycol in humans

The toxicology website <http://toxnet.nlm.nih.gov/> was searched for PG, using terms such as human, aerosol, NOEL, carcinogenicity, inhalation. A review of PG has concluded it is safe for use in cosmetics at concentrations up to 50%.⁶

Absorption. PG vapor has 100% deposition efficiency in human airways.⁷

It is partly absorbed on inhalation. PG is absorbed completely from the gastrointestinal tract and partly and partly through the skin.

Metabolism. It is metabolized to lactic acid and pyruvic acid, and further oxidized to glycogen or carbon dioxide and water. In man, approximately 20-25% of the PG is eliminated unchanged via the kidney.

Inhalational safety in children. In a series of experiments to control airborne infections, over 105 children were subjected to bactericidal concentrations of propylene glycol in the wards of a children's convalescent home in experiments conducted over 3 years.

Method. Six wards of the Children's Seashore House in Atlanta containing 105 bedfast children aged 3 to 15 years were divided into 3 control and 3 undergoing vaporization for 3 week periods with 2 to 3 days between, before the control wards become vaporized, and the vaporized wards became controls. This rotation continued for 7 months. The PG was heated to vaporize it, but not above 80 degrees C, and vaporization continuously maintained a concentration of 0.069 mg per liter. (0.07 ppm)

Results. No ill effects were reported. In the first year, 100 infections occurred in control wards without PG, and 5 in wards with PG vaporization, with rates of 0.18 per week and

⁵ Robertson OH, Loosli CG, Puck TT, Wise H, Lemon HM, Lester Jnr, W. Tests for the chronic toxicity of propylene glycol and triethylene glycol on monkeys and rats by vapour inhalation and oral administration.

J Pharmacol Exper Therapeutics 1947; 91:52-76.

⁶ Anonymous. Final Report on the Safety Assessment of Propylene Glycol and Polypropylene Glycols J Am College of Toxicology. 1994; 13: 437-491. Final draft.

⁷ Soderholm SC, Anderson DA, Utell MJ et al. Method of measuring the total deposition efficiency of volatile aerosols in humans. J. Aerosol Science. 1991; 22: 917-26.

0.09 per week respectively. Most of the upper respiratory infections in control wards were common colds, suggesting the PG is also virucidal.⁸

Inhalational safety in adults. The website www.pneumotox.com devoted to inhalational toxicology, registers one case report of bronchospasm⁹ but no other adverse effects.

Repeated exposure to inhalations of theatrical smoke, including PG, was studied by Varughese and colleagues^{10,11} in 101 employees at 19 sites. They did not report propylene glycol effects separately. They found wheezing and chest tightness correlated with higher cumulative exposure to smoke over the previous 2 years. Glycol fogs in particular were associated with acute cough and dry throat. Those with greater proximity to fog source showed significantly decreased lung function.

Carcinogenicity. There is no evidence that PG is a carcinogen.

PG exposure per puff of the Ruyan® e-cigarette. The cartridge of the Ruyan® e-cigarette contains approximately 1g of PG, of which 0.9 g is extractable from the pad. The concentration of PG in the mouth from one drag of the Ruyan® e-cigarette (900 mg per cartridge, 300 puffs = 3mg) is 3 mg per mouthful.

PG exposure per day of using Ruyan® e-cigarette. If the cartridge lasts 2-3 days as expected, then the inhaled dose is 0.3 to 0.45 g per day, and if used more intensively, could result in 0.9 g of PG inhaled and probably absorbed.

No-observed-effects level (NOEL) and RfD (reference dose) for humans for sub-chronic (less than a lifetime) and chronic inhalational exposure to PG is estimated by US EPA at 116 mg per 70 Kg human. This level, derived from rat studies, allows a safety factor of 100, 10 for inter-species extrapolation, and 10 to allow for susceptible individuals.¹ This NOEL, however, is artificially low – an artefact of PG’s low vapor pressure, as the researchers could not ensure higher concentrations of PG into the air breathed by the rats.

Inhalational Minimal Risk Levels (MRLs). No MRLs for acute- or chronic-duration inhalation exposure to propylene glycol were derived because data are insufficient.¹²

Inhalation threshold. The USEPA has developed no inhalation threshold value for it, nor has Cal/EPA. Inhalation toxicity is not an issue.

⁸ Harris TN and Stokes Jnr, J. Summary of 3-year study of the clinical application of the disinfection of air by glycol vapour. *Am. J. Med Sci.* 1945; 209:152-156.

⁹ Spreux A, Boyer A, Baldin B, et al. Toux et crise d’asthme declenchees par le propylene glycol. Propylene glycol-induced cough or asthma. A case report. *Therapie* 1996 ; 51 : 561-562.

¹⁰ Varughese S, Teschke K, Brauer M, Chow Y, van Netten C, Kennedy SM. Effects of theatrical smokes and fogs on respiratory health in the entertainment industry. *Am J Ind Med.* 2005 May;47(5):411-8.

¹¹ Teschke K, Chow Y, van Netten C, Varughese S, Kennedy SM, Brauer M. Exposures to atmospheric effects in the entertainment industry. *J Occup Environ Hyg.* 2005 May;2(5):277-84.

¹² ATSDR (Agency for Toxic Substances and Disease Registry.) Toxicological profile for ethylene glycol and propylene glycol. Sept 1997. <http://www.atsdr.cdc.gov/toxprofiles/tp96-c2.pdf> at p.108.

2. Tobacco flavor, Nitrosamines and MAO inhibitor effects

2.1 Tobacco flavor

In cartridges dated November and December 2007: The fragrance, odor and taste of tobacco remained. The manufacturer's recipe (Appendix 1) suggested that these attributes came from a flavor base containing tobacco extract weighing 6 mg per cartridge.

In cartridges dated June 2008: The tobacco odor is not obtained.

In both 2007 and 2008 cartridges the cartridge liquid does not behave like tobacco:

Note: Flavors and fragrances have not been tested. See Appendix 1.

2.2 Tobacco-specific nitrosamines

Rationale. Trace levels are found in all nicotine products extracted from tobacco; higher levels suggest the presence of tobacco. Very low levels suggest the e-cigarette cartridge liquid is tobacco free.

Method. Labstat method TWT-333. Determination of tobacco specific nitrosamines (TSNAs) in whole tobacco, modified for the e-cartridges supplied, using LC-MS/MS.

Laboratory. Labstat International ULC, Kingston, Ontario, Canada.

Results. TSNAs, found only in tobacco, were not found in the Ruyan® e-cigarette cartridge liquid except at trace quantity, at a very low level uncharacteristic of tobacco.

Table 2.2 Tobacco specific nitrosamines (TSNAs) in the cartridge liquid of the Ruyan® e-cigarette, November 2007

Nicotine Per Cartridge	Sample ID	NNN (ng/cartridge) Observation	NAT (ng/cartridge) Observation	NAB (ng/cartridge) Observation	NNK (ng/cartridge) Observation	TSNAs
						ng/cartridge total
0 mg	073277	BDL	BDL	NQ	0.260	0.260
6 mg	073278	1.42	1.02	BDL	0.628	3.068
11 mg	073279	1.83	1.36	NQ	1.01	4.200
16 mg	073280	3.87	2.16	0.693	1.46	8.183
Labstat 2007 ¹³ .					Average TSNAs	3.928
BDL = Below the limit of detection. NQ = Not quantifiable. TSNA = tobacco specific nitrosamines. NAB= nitrosoanabasine NNN= nitrosornicotine, NAT= nitrosoanatabine, NNK= 4-nitrosomethylamino-1-(3-pyridyl)-1-butanone						

Comment. 1) Tobacco-specific nitrosamines (TSNAs) were found, equal to 8 ng, in the 1 g of liquid of the 16 mg cartridge. This amount is extremely small, equal for example, to the amount reported to be present in a nicotine medicinal patch. (8 ng in 1g = eight parts per trillion).

2) These very small amounts traces are likely to be due to the fact that even medicinal grade nicotine is extracted from tobacco.

¹³ Rickert W. Determination of Tobacco specific Nitrosamines by LC-MS/MS. Project NZ9. Nov.30, 2007. Labstat International ULC. Kingston Ontario, Canada.

3) The level in the 16 mg nicotine cartridge, for example, is 31 times the level in the 0 mg cartridge.

On a daily dose basis, TSNA's in the 16 mg nicotine e-cartridge are 1200 times less than in the tobacco of 20 manufactured cigarettes, and 3000 times less than the daily dose in a can of Swedish moist snuff.¹⁴

Conclusion. The Ruyan® e-cigarette cartridge does not contain carcinogenic levels of TSNA's, in that no product containing these trace levels has been shown to cause cancer.

2.3 Monoamine oxidase.

Rationale. MAO, an enzyme naturally found in blood platelets and the brain, metabolises dopamine, also known as the pleasure drug. When this process is inhibited by known MAO inhibitors in tobacco smoke, dopamine tends to accumulate, reinforcing the effect of nicotine. The question is whether e-cigarette cartridge liquid also acts to inhibit MAO and reinforce the addictive effect of nicotine, or whether it acts like pure nicotine.

Method. Samples of the liquid contained in the Ruyan e-cigarette cartridges were tested with a monoamine oxidase (MAO) enzyme activity assay which employs the fluorescent MAO substrate kynuramine, and the effect compared with that from tobacco extracts including a nicotine-free tobacco.

Laboratory. ESR Porirua NZ, a Crown Research Institute.

Results. Monoamine oxidase (MAO) enzymes both A and B, were strongly inhibited by tobacco smoke extracts but the cartridge liquid alone had no such effect¹⁵.

Conclusion. The Ruyan E-cigarette cartridge liquid does not behave like a tobacco extract. The absence of a MAO inhibitor effect means the e-cigarette has no detectable addictive potential beyond that of nicotine.

3. Tests conducted

3.1 Direct measurement of e-cigarette mist

Mist can be extracted by syringe from the mouth-end of the e-cigarette, but activation of the electronic micro-circuit requires lip pressure on the mouth end. Unless the circuit is activated, vaporization of nicotine will not occur nor will propylene glycol mist be created. The LED when it lights up at the tip of the e-cigarette, indicates the circuit is activated. But in addition, visible mist of propylene glycol has to be extracted. As the aim is to detect for possible harm, a rich thick mist is preferable.

¹⁴ Wahlberg I. Tobacco-specific nitrosamines in unburnt New Zealand tobaccos. Report to Health New Zealand Ltd. Swedish Match 2004. www.smokeless.org.nz/snuffregulations.htm at Table 2.

¹⁵ Lewis A. Investigation into the effect of RUYAN cartridge exposure on Monoamine oxidase enzyme activity *in vitro*. ESR October 2007.

3.1.1 In e-cigarette mist, analyzed by GC-MS

Rationale. 60 ml was the average puff volume manufactured cigarette smokers smoking four cigarettes each;¹⁶ and until proved otherwise, it is assumed that e-cigarette users will draw the same puff volume.

Method. 60 ml of e-cigarette mist was extracted by gas-tight syringe from an 11mg e-cigarette – mist as would be delivered to the mouth by an e-cigarette user, and connected to a type II ATD (thermal desorption tube) and analyzed qualitatively by GC-MS.

Laboratory. Hill Laboratory, Hamilton, New Zealand.

Results. The mist contained propylene glycol, ethyl alcohol; nicotine and acetaldehyde were minor peaks. A different analysis is required to determine whether this acetaldehyde is artefact or not, as acetaldehyde could have been due to heating of the ethyl alcohol during GC-MS measurement. Other compounds included pyridine and acetone (probably from coffee extract flavoring). Acrolein was not detected.¹⁷

3.1.2 In e-cigarette mist, analysed quantitatively by SIFT-MS¹⁸

Rationale. The SIFT-MS method is well adapted to simultaneously and very accurately analyze the concentration of many volatile gases in real time, once the gases of interest are known. Most of the toxicity of cigarette smoke is due to volatiles.

Method. A disposable plastic syringe (nominally 30 and 60 ml; see below) was connected to the e-Cigarette using a short length of plastic tubing (less than 5 cm long). A moderately rapid pull on the syringe plunger was used to simulate a smoker's puff and hence actuate the e-Cigarette's heating system to volatilize the solvent and dissolved substances, such as nicotine.

The entire contents of the syringe were then injected into a Tedlar sampling bag filled with a specific volume of laboratory air (nominally 1 and 3 L; see Results section). The bag was then analyzed using a Syft Technologies Voice200(R) SIFT-MS instrument.

1. *38 milliliter puff.* In the first and third experiment, a 30-ml syringe was used. When fully extended, this corresponds to a puff of approximately 38 ml. This sample was then injected into one liter of air in a 1-L Tedlar sampling bag.

2. *58 ml puff.* In the second experiment, a 60-ml syringe was used. A 58 to 60 ml puff was taken in the same way as for the 38-ml puff, and then injected into three liters of air in a 3-L Tedlar sampling bag, before analysis.

¹⁶ Laugesen M, Epton M, Frampton C, et al. Toxicity comparison of roll-your-own and factory-made cigarettes. In preparation.

¹⁷ Graves I. Report no. 468304. 60 ml sample of mist from 11 mg nicotine e-cigarette cartridge. Thermal desorption tubes. Hill Laboratories. Hamilton New Zealand, 5 September 2008.

¹⁸ Langford V. SIFT-MS Analysis of Ruyan e-cigarette Nicotine Cartridges. Syft Analytics. October 2008

Analytes. The toxicants selected for study were those identified by Fowles and Dybing in 2003¹⁹ as the top priority volatile toxicants in cigarette smoke. To these have been added some compounds of interest which are chemically related to propylene glycol. As listed in Table 3.1.2 below.

Laboratory. Syft Analytics Ltd, Christchurch, NZ.

Results.

Three experiments were undertaken, as follows:

1. 38 millilitre puff. After correcting for dilution, the most common cigarette smoke toxicants were found to be either completely absent, or present in amounts less than 1 part per million. The LED at the tip of the e-cigarette was activated, indicating air was pulled through the e-cigarette. The mean results from analysis of duplicate samples were as follows:

Table 3.1.2 Compounds measured in first 38 ml sample of e-cigarette mist.

Compound	Mean	Units
1,3-butadiene	not detected	Ppm
Acetaldehyde	0.34	Ppm
Acetone	0.16	Ppm
Acrolein	not detected	Ppm
Acrylonitrile	not detected	Ppm
Benzene	not detected	Ppm
Ethanol	100	Ppm
Ethylene glycol	not detected	Ppm
Ethylene oxide	not detected	Ppm
Formaldehyde	0.25	Ppm
Hydrogen cyanide	not detected	Ppm
Cresol	0.16	Ppm
Xylene	0.18	Ppm
Nicotine	not detected	Ppm
Propylene glycol	32	Ppm
Propylene oxide	not detected	Ppm
Styrene	0.29	Ppm

Note: The limit of detection was 0.01 ppm and the limit of quantification was 0.025 ppm for this analysis.

2. 58 milliliter puff. Analysis revealed that the concentration of propylene glycol was too high for the SIFT-MS instrument, so a subsequent dilution was made bringing the overall dilution to 1 part puff in 1500 parts air. The results obtained revealed a concentration of approximately 0.5% propylene glycol (5000 ppm) in the original puff, but did not allow the researchers to gain any reliable quantitative data for the toxicants.

¹⁹ J Fowles and E Dybing. Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. Tob. Control, Dec 2003; 12: 424 - 430.

3. 38 millilitre puffs revisited with a new cartridge

A 38 mL puff was taken as previously described. With the new cartridge, it was found that the results were similar to those obtained for the 58 mL puffs on the used cartridge. That is, with the new cartridge the solvent was volatilised much more readily, yielding approximately 0.3% propylene glycol (3000 ppm) in the vapour for five replicate samples made over a ten-minute period. The high propylene glycol concentration again prevented reliable quantitative data being determined for any of the toxicants, as per experiment 2 above.

Comment The two experiments using different puff sizes very clearly demonstrate that only the latter part of the puffs results in a substantial dosing of the user with propylene glycol (and hence nicotine) when a well-used cartridge is puffed.. It seems the user will get little benefit from a short puff. Presumably this is due to the time taken to heat the cartridge. However, the third experiment showed that a new cartridge will deliver a more satisfactory dose of propylene glycol with a smaller puff than will an identical puff with an older (ie previously opened) cartridge. Further work is needed to clarify whether this is indeed due to difference in puff volume, or to flow velocity, or to number of puffs from the beginning of the session.

Unfortunately, the second and third experiments, which resulted in very high propylene glycol concentrations, reveal that SIFT-MS cannot undertake measurements of samples that exactly mimic a smoker's puff. This is because SIFT-MS cannot obtain reliable measurements for trace compounds in the presence of overwhelming quantities of solvent (propylene glycol in this case).

3.2 Measurement of the volatiles in the headspace above the cartridge liquid.

This method records the volatiles that can be elicited at room temperature in the just opened e-cigarette cartridge.

3.2.1 Using Head Space Solid-Phase Micro-Extraction (HS-SPME) and GC-MS²⁰

Introduction. Head Space Solid-Phase Micro-Extraction (HS-SPME) was the sampling technique used to sample the headspace volatiles emitted from the sample upon heating. This involved exposing a conditioned fiber into the headspace of a sealed vial and allowing the volatile compounds in the headspace to absorb onto the fiber surfaces. These volatile compounds were then introduced into the GCMS by exposing the fiber inside the GC injection port where they were stripped off at a high temperature. The compounds detected by the mass spectrometer were Qualified only, i.e. identified by comparison with a mass spectral library and their relative abundances reported. Concentrations for these compounds were not obtained. In order to obtain concentration information the protocol used would need to be changed to include the use of standards, both internal and external.

Method. Samples were analyzed using a Shimadzu GCMS-QP2010 gas chromatograph mass spectrometer fitted with a Restek Rtx-WAX fused silica capillary column (30.0m x 0.25mm i.d. x 0.50µm film thickness) coupled in series with a Restek Rtx-1ms fused silica capillary column (15m x 0.25mm id x 0.25µm film thickness).

²⁰ Sherlock R. Lincoln. Lincoln University, Soil and Physical Sciences Group. www.lincoln.ac.nz

Sample preparation involved placing the e-cigarette into a 20 ml SPME sample vial where it was then quickly capped. Using a CTC-Combi PAL auto sampler (Shimadzu AOC-5000), samples were incubated for 60 min at 37°C with their enclosed headspace exposed to a 2 cm long DVB/CAR/PDMS combination SPME fiber (Supelco). During this exposure period the headspace volatiles were absorbed onto the fiber.

Desorption of these volatiles occurred when the SPME fibre was inserted (by the Autosampler) into the heated (250 deg C) injection port of the Shimadzu GCMS-QP2010 gas chromatograph–mass spectrometer. The injection port was then used in Splitless mode operating with a Helium carrier gas linear flow of 25.9cm/s (column flow). The GC columns were held initially at 35 deg C for 5mins, ramped to 100 deg C at 7 deg C/min where it was then ramped to 200 deg C at 3 deg C/min, and then finally ramped to 250 deg C at 7 deg C /min and held for 10mins.

During the elution of the compounds the GC–MS was operated in scan mode at a detector voltage of 1.2kV and electron impact ionisation voltage of 70 eV. All compounds detected were identified by matching their mass spectra with the spectra of reference compounds found in the NIST EPA/NIH Mass Spectral Library database (National Institute of Standards and Technology, NIST05).

Laboratory. Lincoln University, Lincoln, Canterbury region, New Zealand.

Results For a graphic of the Feb. 2008 run result, also see www.healthnz.co.nz/Portland2008ECIG.pdf The table below summarizes the result. The SIFT-MS tests on mist are still to be completed.

Table 3.2.1 Screening of cartridge headspace vapor and mist from the e-cigarette (date of manufacture label June 2008) by different methods

Compound	In headspace vapor of Ruyan® e-cartridge Detected YES or NO		In mist of Ruyan® e-cigarette
	HS-SPME Detected or not 37deg C	SIFT-MS Mass Screen; ppm, 37deg C. Quantitative	ATD- GCMS Screen Qualitative
Laboratory	Lincoln University	Syft Analytics Ltd	Hill Laboratories Ltd
Main constituents			
Propylene glycol	YES	YES	YES
Nicotine	YES	Not detected.	YES
Alcohol	YES	YES +++	YES
Other volatiles			
Acetaldehyde	YES	YES 5.1 ppm	YES
Acetone	YES##	Not detected.	YES##
Acrolein	NO	YES 0.33 ppm	NO

Acrylonitrile	YES	BLQ*.	NO
Benzene	NO	BLQ*	NO
1,3, Butadiene	NO.	BLQ*	NO
m-, o-, p- Cresols	NO	BLQ*	NO
Ethylene oxide	NO	Not detected.	NO
Hydrogen cyanide	NO	BLQ*	NO
Styrene	YES	Not detected.	NO
Xylenes	YES	Not detected.	NO

*BLQ – If present, the quantity is below the limit of quantification of 0.3 ppm or below.).

in background air.

Not detected: Either absent, or present in a concentration less than the limit of detection (0.01 parts per million.)

Langford 2008²¹

3.2.2 By SIFT-MS (Selected Ion Flow Tube and Mass Spectrograph) method

Aim. To test the headspace of liquid from freshly opened (un-smoked) cartridges after incubation for one hour at 37 deg C, by SIFT-MS method.

Method. Ruyan e-Cigarette cartridges (16 mg nicotine; batch 20071228, and 6 mg nicotine, batch 20080627) had their wisp removed and one wisp was placed in each of two 500-ml glass Schott bottles, which were then capped with pierceable septa. Duplicate blank samples of laboratory air were also analyzed for comparison. Bottles were then incubated at 37 °C for approximately 60 minutes prior to analysis.

SIFT-MS analyses gas samples for volatile organic compounds (VOCs) and certain inorganic compounds.^{22 23} Typically it can accurately detect and quantify these compounds in real time at very low concentrations (usually to parts-per-trillion {ppt} levels), even at breath humidity. SIFT-MS does not employ chromatographic separation and hence cannot perform well when high levels of organic solvents are present. A Syft Technologies Voice100TM instrument was used for this work.

Laboratory. Syft Analytics Ltd, Christchurch NZ.

Results. Table 3.2.2 shows results of a revised formulation of the cartridge liquid tested in June 2008. Concentrations of all four gases found in the cartridges tested in February

²¹ Langford V. SIFT-MS Headspace Analysis of Nicotine Cartridges from Ruyan e-Cigarettes. Christchurch. SYFT Ltd. February 2008.

²² C.G. Freeman and M.J. McEwan (2002). "Rapid analysis of trace gases in complex mixtures using Selected Ion Flow Tube–Mass Spectrometry." *Australian Journal of Chemistry*, 55, 491-494.

²³ D. Smith and P. Spanel (2005). "Selected ion flow tube mass spectrometry (SIFT-MS) for on-line trace gas analysis." *Mass Spectrometry Reviews*, 24, 661-700.

2008 - acetaldehyde, acrolein, benzene and cresols decreased, or were now present in such small quantities that although detectable as being present, were not measurable.

Table 3.2.2 SIFT-MS headspace analysis of the Ruyan® e-cigarette cartridge (mean of two replicates).

Toxicant	Net Concentration in headspace of cartridge ppm by volume))	
Date of manufacture	December 2007	June 2008
Date of test	February 2008	August 2008
Acetaldehyde	9.2	5.1
Benzene	1.2	BLQ
1,3-Butadiene	BLQ	Not detected
Hydrogen cyanide	BLQ	Not detected
Acrolein	1.00	<0.33
Acrylonitrile	BLQ	Not detected
Cresols (total m-, o- and p-)	0.19	BLQ
Propylene oxide	BLQ	Not detected
Diethylene oxide	BLQ	Not detected

Langford 2008²⁴ Limit of quantification = 0.3 ppm. BLQ means the upper limit is 0.3 ppm. Not detected: Either absent, or present in a concentration less than the limit of detection (0.01 parts per million.)

Note: After the February tests of the December manufactured cartridges showed presence of benzene, albeit at low levels, Ruyan conducted independent tests to determine the exact source of this contaminant. Sources of Propylene Glycol, Nicotine, and each flavoring were tested separately. These tests determined that the source of the contaminant was due to the flavoring. The formula of the flavoring was changed and the updated Cartridges manufactured in June and tested in August shows no detectable level of benzene.

Comment: The concentrations in Table 3.2.2 cannot be converted to micrograms of toxicant in the cartridge. The concentration in inhaled mist is likely to be lower, because with each puff only a minute quantity of cartridge liquid will be vaporized, and greatly diluted by inhaled air. High levels of ethanol were found in the cartridges (identified from the full scans). This meant that the SIFT-MS instrument had to be run at reduced sensitivity for the analysis presented here, with a degraded (higher) limit of quantification (LOQ = 300 ppb). Consequently, some target compounds could not be reported, as all their available ion products suffered significant interference; and for the toxicants reported, the results represent an upper limit to the true concentration in the wisp.

²⁴ Langford V. SIFT-MS Headspace Analysis of Nicotine Cartridges from Ruyan e-Cigarettes. Christchurch. SYFT Ltd. February 2008.

- Using SIFT-MS, due to interference from alcohol in the Ruyan cartridge, ethylene oxide could not be separated from acetaldehyde. Using HS SPME method, however, ethylene oxide was not detected in the headspace of the Ruyan cartridge, and therefore 5700 ppb (5.1 ppm net) is due to acetaldehyde.
- Acrylonitrile does not register: no response was obtained. Although below the level of quantification of 0.3 ppm, it suggests that acrylonitrile is absent from headspace vapor. As it is absent in the mist (Table 3.2.1).

3.3 Measurement of the Ruyan® e-cigarette cartridge liquid.

3.3.1 Polycyclic Aromatic Hydrocarbons

Method. The liquid from 5 unopened cartridges of 0 mg nicotine dated 21 November 2007 were analyzed by extraction with hexane, then partitioned against a solution of potassium carbonate, and dried by passing through a column of sodium sulphate. The concentrated extract was purified by absorption chromatography, followed by gel permeation chromatography, then concentrated and transferred into iso-octane for analysis by GCMS. PAHs were identified by single ion monitoring and quantified against calibration standards prepared from certified analytical standards, using internal standard calibration.

Laboratory. Hort Research Food and Biological Chemistry Laboratory, Hamilton NZ.

Results. The absolute mass of 34 PAH compounds in the extract of cartridge liquid was measured separately in nanograms. A number of lighter PAHs are present in this extract but it does not contain the higher molecular weight and carcinogenic PAHs such as the benzopyrenes. The PAH content is dominated by fluoranthene and pyrene, the PAHs most commonly detected as combustion residues, and by naphthalenes not listed as cigarette smoke carcinogens.

The concentration of the detected PAHs is relatively constant between the replicate samples that were analyzed, suggesting a common source of ingredient.

Of the 34 PAHs tested, only those rated and listed as carcinogens by the International Agency for Research on Cancer and Californian Environmental Protection Agency, are shown in Table 3.3.1. Four of these carcinogens were detected in both the cartridge liquid, and in the list of the California Environment Protection Agency as cigarette smoke carcinogens. These four carcinogens are not classified as human carcinogens, and do not have any listed cancer potency ratings.

Comment: In Table 3.3.1, e-cigarette consumption was assumed to be equal to 20 tobacco cigarettes (one day's use). In fact users may take up to 4 days to exhaust one Ruyan e-cigarette cartridge. The maximum likely dose by e-cigarette for Anthracene, Phenanthrene, 1-Methyl phenanthrene and Pyrene which are not classifiable as carcinogens was no more than 1 percent of the amount obtained from 20 cigarettes.

Conclusion. PAH carcinogens found in cigarette smoke are not detectable in the Ruyan e-cigarette cartridge liquid. PAHs that were detected are not rated as carcinogens by IARC. In particular, the cigarette smoke carcinogen benzalaphapyrene was not detected.²⁵

Table 3.3.1. Polycyclic Aromatic Hydrocarbons in Ruyan e-cigarette cartridge, 0 mg nicotine.

Compound	Mean of 5 samples tested. Absolute mass	Mean value for mainstream smoke of one manufactured cigarette ²⁶ . Absolute mass.	IARC ²⁷ Carcinogenic status	PAH in e-cigarette liquid as % of amount in smoke of an equivalent number of tobacco cigarettes
<i>Unit</i>	<i>ng</i>	<i>Ng</i>	<i>Group</i>	<i>%</i>
Anthracene	7	130	3	0.3
Phenanthrene	48	350	3	0.7
1-Methyl phenanthrene	5	30	3	0.9
Pyrene	36	130	3	1.4
Benz(a) anthracene	nd	45	2A	0
Chrysene	nd	50	3	0
Benzo(b) fluoranthene	nd	30	2B	0
Benzo(k) fluoranthene	nd	9	2B	0
Benzo(a) pyrene	nd	35	2A	0
Benzo(e) pyrene	nd	16	3	0
Perylene	nd	4	3	0
Indeno(1,2,3-cd)pyrene	nd	12	2B	0
Dibenz(ah)anthracene	nd	4	2A	0
Benzo(ghi) perylene	nd	60	3	0

Human carcinogenicity rating from International Agency for Research on Cancer (IARC) : 2A = probable; 2B = Possible; Group 3 =Not classifiable as carcinogen. 1 ng = one billionth of a gram.

²⁵ Benzoalpha pyrene. Hort Research Report to ESR 19 November 2007.

²⁶ Fowles J, Dybing E. Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. Tobacco Control 2003; 12:424-430, at web Table 1.

²⁷ International Agency for Research on Cancer. Ratings given at web Table 1 as above.

3.3.2 Heavy metals

Rationale. Heavy metals such as chromium, arsenic, and nickel can cause cancer, and lead is a neuro-toxicant.

Laboratory. Environmental Science Research, Porirua, Wellington Region, NZ

Method. Inductively coupled plasma mass spectrometry (ICP-MS), following APHA 21st edition method 3125 modified.

The liquid was tested for heavy metals (Arsenic, Antimony, Cadmium, Chromium, Cobalt, Copper, Lead, Manganese and Nickel).

Results. The e-cigarette cartridge liquid contains none of the above heavy metals. No metals were detected above the limit of detection for each metal, as given in Table 3.3.2. In contrast, heavy metals have been found in low-nitrosamine Swedish snus and in unburnt factory-made cigarettes and cigarette tobacco.

Table 3.3.2. Heavy metal concentrations, e-cigarette cartridge liquid.

		As	Cd	Chr	Ni	Pb
Ruyan e-cigarette liquid 2008 ESR. (ug per g)	Daily (from 300 puffs, or one cartridge)	ND	ND	ND	ND	ND

Limit of Detection, As <0.1ppm, Cd <0.01ppm, Chr <0.2ppm, Ni <0.2ppm, Pb <0.1ppm.

Environmental Science and Research. (ESR) Report to Health New Zealand Ltd. One cartridge is taken as an upper limit of usual daily consumption.

3.3.3 Other possibly hazardous substances

Rationale. These were selected for testing as possibly derived from propylene glycol.

Method. GCMS (gas chromatograph, mass spectrograph) testing.

Laboratory. Environmental Science Research (ESR), Porirua, New Zealand.

Results. Propylene oxide and ethylene oxide were not detected above the limit of detection (16.75 ug/ml and 42.5 ug/ml respectively). Some interference (matrix effect) prevented accurate quantification.²⁸ However neither compound was detected by the HP-SPME scan, suggesting their levels if present were likely to be under 1 ppm. To avoid this technical problem, a different method of analysis was then used. For these additional results, see 3.1.2.

3.4 The cartridge as a whole – Radioactivity

Rationale. Isotopes of lead have been found in tobacco smoke extracts.

Method. Two 16 mg cartridges for Ruyan V8 e-cigarette were tested by gamma spectroscopy for Pb 210 at the National Radiation Laboratory of New Zealand.

²⁸ Fitzmaurice P. Testing of Ruyan E-cigarette cartridges for ethylene oxide and propylene oxide content. 18 December 2007. Environmental Science Research. Porirua, Wellington region, New Zealand..

Laboratory. National Radiation Laboratory of New Zealand, Christchurch.

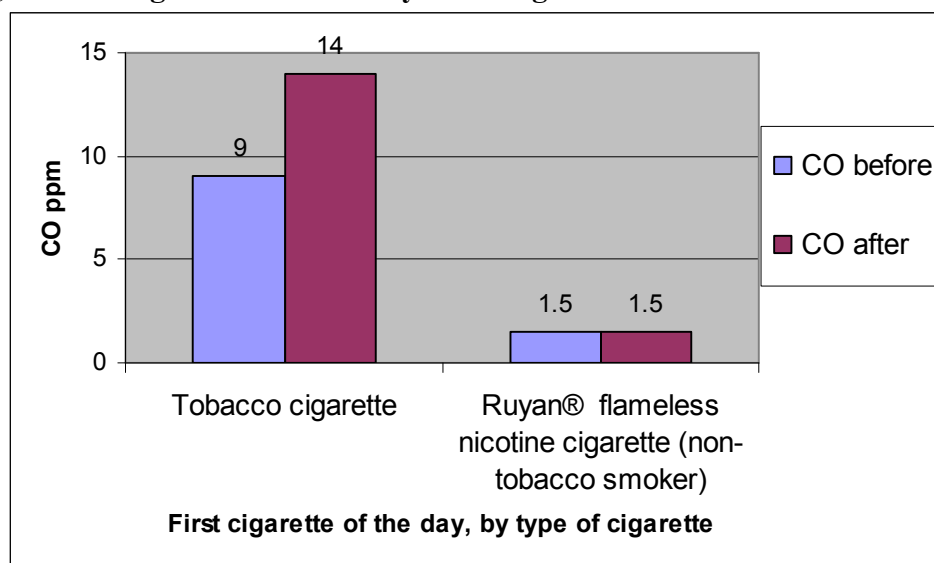
Results. Lead-210 Bq/unit was <0.012. No gamma-emitting nucleotides were found to be above the detection limit.²⁹

3.5 Measurement of the exhaled breath after using the Ruyan® e-cigarette

Rationale. Carbon monoxide is a product of combustion and therefore can distinguish between the smoke produced by burning tobacco versus flameless cigarettes.

Method. Five minutes after their final puff of their first cigarette of the day, 48 smokers exhaled into a MicroMedical CO analyzer.³⁰ A non-tobacco smoker with a smokefree home and workplace, was similarly tested after taking 20 lung inhalations from the Ruyan® e-cigarette. The volunteers held their breath for 20 seconds before exhaling – to allow for full mixing of the CO in inhaled air and in the lung alveoli. The measured concentration of CO in exhaled breath reflects the CO concentration in the alveoli, and in the blood. The increased from before to after smoking reflects changes in CO concentration due to the cigarette used.

Figure 3.5 Carbon monoxide in exhaled breath, before and after the first cigarette of the day, tobacco cigarettes versus Ruyan® e-cigarette



Clinic. Canterbury Respiratory Research Group, University of Otago, Christchurch, and Health New Zealand Ltd, Christchurch..

Results. The tobacco cigarette boosted CO in exhaled breath by an average of 5 ppm, but did not increase it at all in the non-smoker inhaling from the Ruyan® e-cigarette.

²⁹ Decallion J-G. National Radiation Laboratory Report 2008-29. 24 April 2008. Christchurch New Zealand.

³⁰ MicroMedical CO analyzer. sales@micromedical.co.uk.

Interpretation

Cigarette smoke. The presence of CO indicates combustion of organic compounds, and so is found in all smoke involving burning of tobacco, or any other plant material, such as wood smoke and marijuana smoke.

The e-cigarette. Shows no increase in expired CO. The absence of any such effect from the e-cigarette shows that combustion does not occur – as confirmed by the lack of flame or smoke. As nicotine has a low vapor pressure, the piezoelectric ceramic element in the e-cigarette is needed to cause vaporization of the nicotine-propylene glycol solution. Cigarette smoke is produced by combustion at temperatures of up to 1000 degrees Centigrade, which is highly destructive, breaking up tobacco into free radicals and many small harmful gas molecules such as carbon monoxide, butadiene, benzene etc.

Conclusion

Mist from the e-cigarette is created by vaporizing a liquid, whereas smoke is created by incinerating plant material. Mist and smoke differ profoundly in composition and safety profile.

Overall conclusion

Several toxicants in headspace of the Ruyan® e-cigarette cartridge have, on some tests, been found, specifically acrolein and acetaldehyde, at very low levels, and at levels below those determined to be harmful, and well below the minimum risk levels accepted by the US Public Health Service and OSHA.

The results obtained to date do not mitigate this report's overall conclusion that the Ruyan® e-cigarette is designed to be a safe alternative to smoking, and appears to be safe in absolute terms on all measurements we have applied.

30 October 2008

4. Risk of cross-infection from use

4.1 Risk of cross-infection from the mouthpiece or by inhalation

Inter-user transmission of bacterial or virus via the mouthpiece surface is a possibility, and two-way flow of air can occur through the e-cigarette.

On the other hand, propylene glycol vapor is bactericidal, and virucidal against airborne aerosol particles, and is associated with reduced respiratory infections in children continually exposed to propylene glycol vapor.³¹

³¹ Robertson OH, Bigg E, Puck TT Miller BF. The bactericidal action of propylene glycol vapor on microorganisms suspended in air 1. J. Experimental Medicine 1942; 75: 593-610.

Conclusion. Public health agencies typically advise people not to share drinking glasses or cigarettes, due to the risk of cross-infection from lip saliva on the mouth end. This advice should be extended to e-cigarettes. Manufacturers' instructions to users should discourage cigarette sharing because of the risk of transfer of meningococcal meningitis, tuberculosis and other infectious diseases.

4.2 Risk of micro-organisms in the cartridge liquid

Rationale. Another risk would be if the liquid in the cartridge acted as a culture medium for micro-organisms, despite the 90% propylene glycol and 5% alcohol content of the cartridge liquid (Appendix 1).

Laboratory. Environmental Science Research, Porirua, Wellington, NZ.

Method. Tested one used and one unused Ruyan® cartridge for the presence of the three main classes of micro-organism (aerobic, anaerobic and Legionella)³². None was found.

Conclusion. There is no inherent tendency in the design of the Ruyan® e-cigarette towards contamination from growth of organisms in the cartridge liquid.

5. Safety of Ruyan® e-cigarette 'smoke' for bystanders.

Method. Analysis of published data on nicotine absorption, and informal comments of bystanders, and observation of e-cigarette smoking indoors.

Results. Cigarette smoke is a mixture of sidestream smoke and exhaled mainstream smoke. In contrast, the e-cigarette generates no sidestream smoke from its (artificially lit) tip. Any exhaled PG mist visibly dissipates to vapor within seconds. Non-smoking bystanders do not find the mist unpleasant. The mist is odorless, and those close by quickly realize it does not have the odor of smoke or the irritating quality of tobacco cigarette smoke.

Comments. Inhaled nicotine in cigarette smoke is over 98% absorbed⁶, and so the exhaled mist of the e-cigarette is composed of propylene glycol, and probably contains almost no nicotine; and no CO. (see Figure 3.5) Lacking any active ingredient or any gaseous products of combustion, the PG mist or 'smoke' is not harmful to bystanders. The 'smoke' or mist is not tobacco smoke, and not from combustion – no flame is lit – and is not defined as environmental tobacco smoke. E-cigarette "smoking" would be permitted under New Zealand's Smoke-free Environments Act 1990.³³

³² Analytical Report no.07/15857. ESR Kenepuru Science Centre, Porirua NZ. 6 September 2007.

³³ Johnston M. No smoke, no fire, just nicotine. NZ Herald 8 December 2007, quoting Dr Ashley Bloomfield, Chief Advisor, Public Health, New Zealand Ministry of Health.

Appendix 1. Composition of cartridge liquid in the Ruyan® e-cigarette

Summary:

Based on the manufacturer's information, the composition of the cartridge liquid is not hazardous to health, if used as intended.

Table 1.1: Chemical compositions (quantity) released from each Ruyan® cartridge

Chemical content released from each cartridge	Cartridge Specification, named by nicotine content			
	16mg	11mg	6mg	0mg
Water (mg)	40	40	40	40
Alcohol (mg)	50	50	50	50
Propylene glycerol (mg)	888	893	898	904
Nicotine (mg)	16	11	6	0
Flavor Base (mg) *	6	6	6	6
Total (mg)	1000	1000	1000	1000

Source: Manufacturer's data

Table 1.2: Chemical compositions (percentage w/w) released from each cartridge

Chemical content released from each cartridge	Cartridge Specification, named by nicotine content.			
	16mg	11mg	6mg	0mg
Water	4%	4%	4%	4%
Alcohol	5%	5%	5%	5%
Propylene glycerol***	88.8%	89.3%	89.8%	90.4%
Nicotine	1.6%	1.1%	0.6%	0.0%
Flavor base *	0.6%	0.6%	0.6%	0.6%
Total	100%	100%	100%	100%

Source: Table 1.1.

*** See section on Propylene Glycol.

*Safety Evaluation: 4-hydroxy-2, 5-dimethyl-3(2H)-furanone and Acetyl pyrazine

1). 4-hydroxy-2, 5-dimethyl-3(2H)-furanone

4-Hydroxy-2, 5-dimethyl-3(2H)-furanone (FEMA 3174, CoE 536) is naturally occurring in various foods and plays an important role in the flavor of numerous fruits as well as in roasted products. 4-hydroxy-2,5-dimethyl-3(2H)-furanone has the odor and taste of fruity, caramelized pineapple-strawberry and is widely used in fresh bread, butter, chocolate, chocolate cocoa, coffee, meat roasted and nut almond.

Over 90% of annual production volume of tetrahydrofuran and furanone flavoring agents is 4-hydroxy-2, 5-dimethyl-3(2H)-furanone. The estimated daily *per capita* intake is 5300 µg in Europe and 5200µg in the USA. Due to the large consumption, the safety of 4-hydroxy-2, 5-dimethyl-3(2H)-furanone is extensively investigated. The oral LD₅₀ for

mouse is 1,608mg/kg. Genotoxicity is observed at high dose, but it is related to a mechanism involving reactive oxygen species, rather than the generation of an active metabolite. A 2-year study in which rat were given a dose up to 400mg/kg bw from diet daily showed no evidence of carcinogenicity. Considering the fact that NOEL of 200mg/kg bw in rat is >2300 times the daily intake as a flavoring agent, the WHO Committee on Food Additives concludes that “the safety of this agent would not be a concern at the estimated current intake”¹.

2). Acetyl pyrazine

Acetyl pyrazine (2-acetyl pyrazine, FEMA 3126, CoE 2286, molecular weight 122, C₆H₆N₂O) vaporizes at 78 °C. At 0.1% in propylene glycol is detectable as a popcorn or coffee odor.³⁴ It is found in beef, coffee, popcorn, sesame seed, almond, wheat bread, cocoa, peanut, pork and potato chips, etc. Acetyl pyrazine belongs to a group of 41 flavoring agents consisting of pyrazine and pyrazine derivatives. Among them, acetyl pyrazine is detected naturally and its daily intake threshold for humans is 540mg/day. The estimated annual consumption of acetyl pyrazine is 920kg in the USA, corresponding to 120µg/person per day. In Europe, the intake of acetyl pyrazine is 14µg/person per day. The consumption of the parent substance pyrazine from food is about 36,000 times greater than its intake as a flavoring agent². Compared to the 540mg/day human intake threshold, the amount is much lower and it is not a safety concern³.

Toxicity data support the above conclusion. In an acute toxicity test on rat, LD₅₀ through gavage was >3,000mg/kg. A group of 32 Wistar rats were maintained on diets containing acetyl pyrazine 8.2mg/kg bw for 90 days. Control group was given basic diet. At the end of experiment, measurements of growth rate, food intake, haematological and clinical chemical parameters, organ weights, and gross and histopathological appearance showed no differences between test and control animals⁴.

Conclusion. Based on the manufacturer’s information, the composition of the cartridge liquid is not hazardous to health, if used as intended.

References

1. WHO Technical Report Series 928: Evaluation of Certain Food Additives, Geneva, 8-17 June 2004.
2. Stofberg, J. & Kirschman, J.C. (1985) The consumption ratio of flavouring materials: A mechanism for setting priorities for safety evaluations. *Food Chem. Toxicol.*, **23**, 857–860.
3. WHO food additives series 48: Safety Evaluation of certain additives and contaminants-pyrazine derivatives.
4. Posternak, J.M., Dufour, J.J., Rogg, C. & Vodoz, C.A. (1975) Summaries of toxicological data: Toxicological tests on flavouring matters. II. Pyrazines and other compounds. *Food Cosmet. Toxicol.*, **13**, 487–490.