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Second Safety Report on the Ruyan® e-cigarette

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Foreword

This report is entitled a second report, and further test results will be added as they come to hand. 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.

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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9 April 2008.

Summary

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

Safety The Ruyan® e-cigarette is designed to be a safe alternative to smoking, and on examination from a number of aspects, appears to very safe relative to cigarettes, and also safe in absolute terms on all measurements we have applied. Using micro-electronics it vapourizes, 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.

By May 2008 at latest, we intend to release results of our study of efficacy of the e-cigarette in raising nicotine blood levels and in relieving cigarette cravings. That study was of smokers using the e-cigarette for the first time, without prior experience of its use. **By June or July 2008**, we plan another edition of this report, in response to findings to date. Upgrade of the cartridge liquid is planned to eliminate traces of contaminants.

Once on sale, its on-going safety profile depends on 1) good manufacturing practice and pharmaceutical-grade purity of the nicotine and propylene glycol used in the cartridge liquid. 2) the prevention of shared use which could result in cross infection.

- A number of e-cigarettes are on sale on the internet from China. This report is specific for the Ruyan® e-cigarette, manufactured by Ruyan (Holdings) Co. Ltd, Hong Kong and Beijing, who invented it, and hold the required patents.

1 Background

1.1 Risks of smoking

According to the World Health Organization, the annual death toll from tobacco smoking was 7.6 million world-wide in 2000, and rising.¹ Globally, smoking will soon exceed AIDS-HIV as the leading preventable cause of death.

Smoking multiplies the risk of dying early, doubling the risk for those who smoke 5 to 9 cigarettes a day, tripling the risk for smokers of 20 cigarettes a day, quadrupling the risk for smokers of over 25 per day.²

1.2 Separating nicotine from the smoke

Smokers smoke for nicotine but do not die from the nicotine³ – they die from the smoke. *Smoking kills*, the warning on cigarette packets in many countries, is a precisely accurate statement.

Smoking kills because tobacco smoke contains cancer-causing tar solids (visible particles) in smoke, and certain known invisible toxicant gases such as butadiene (cancer-causing); hydrogen cyanide and carbon monoxide (affecting heart and blood vessels); and acrolein (damaging to the lungs).

Smoking tobacco is, until now, the only way to inhale nicotine into the lungs. The invention of the Ruyan® nicotine e-cigarette in 2004 is about to change that. The Ruyan® e-cigarette takes advantage of the fact that inhalation via a cigarette is the fastest route for nicotine absorption, and absorption by this route is 99% complete.

Before cigarettes were invented, lung cancer was unknown. People sniffed tobacco in the form of nasal snuff, or sucked or chewed it as oral snuff, instead of smoking it. Pharmacies today stock a range of nicotine products. Nicotine from patches is slowly and completely absorbed through the skin. The mouth mucosa filters out 60% of the nicotine in gum, lozenges and tablets, and absorption through the mouth can take half an hour. None of these methods allows the smoker to continue to enjoy the sensation of drawing on a cigarette to get the nicotine.

1.3 Stopping smoking only way to prevent smoking deaths in next 20 years

- As almost all tobacco smoking deaths occur at age 35 years onwards,⁴ those smokers who will die of smoking in the next 20 years, are already smokers – and their deaths can only be averted if they can be persuaded to stop smoking. Stop

¹ Tobacco Atlas. World Health Organization. www.who.int/tobacco/en/atlas1.1.pdf

² Bjartveit K, Tverdal A. Health consequences of smoking 1-4 cigarettes per day. *Tobacco Control* 2005; 14: 315-20, based on follow-up of 43,000 Norwegians from 1970s to 2002.

³ Murray RP, et al. Safety of nicotine polacrilex gum used by 3094 participants in the Lung Health Study. *Chest* 1996; 109: 438-45. Followed for 5 years, compared with 1900 controls. No increase in hospitalization or mortality was found in the nicotine gum chewers, whether still smoking or not.

- smoking can be either by quitting smoking entirely, or switching to a non-tobacco smoking product.
- Unfortunately, even world-leading programmes to reduce smoking (such as New Zealand's) are succeeding only slowly, so that, by promoting quitting alone, smoking will take another 70 years to reach zero.
 - A large part of the problem is that many smokers are unwilling to quit their addiction to nicotine. The Ruyan® e-cigarette provides an easier escape route for smokers.
 - WHO has recommended that alongside the individual approach (including pharmacological interventions), a supportive (policy) environment is needed, and recommends “a broad framework for addressing smoking cessation and treatment of tobacco dependence.”⁴
 - Such a framework would logically permit widespread sale of a range of cigarette substitutes that each provided “clean” nicotine for lung inhalation.

1.4 Life years reclaimed if smokers switch to smoking the Ruyan® e-cigarette

Here we estimate the public health benefits of widespread adoption of the Ruyan® e-cigarette *or any other product, policy or programme* that can likewise persuade smokers to stop smoking.

At personal level. For every two continuing smokers, one will die early from smoking (on average 13 years early⁵). So if two smokers both switch to Ruyan® e-cigarettes from the beginning, or otherwise succeed in quitting smoking, then 13 life years will be reclaimed.

In percentage terms. Similarly, for every 100 continuing smokers, 50 will die early from their smoking (on average dying 13 years early)⁶ If, however, all 100 switch to e-cigarettes (or otherwise stop smoking tobacco) before 35 years of age, we would expect that 50 fewer will die early, a total of 650 life-years reclaimed, per 100 smokers. This is based on the proven zero excess mortality effect from daily use for five years of nicotine without tobacco.

At country level For New Zealand, with 21% of adults smoking and 656 000 daily smokers,⁷ 4.3 million life years would be saved, in country of 4.2 million population, or one life-year reclaimed per capita, if everyone stopped smoking; equal to increasing life expectancy by one year averaged over the entire population. In reality, it is the smokers who stop smoking by abstinence or switching to the Ruyan® e-cigarette, who obtain this gain in longer life.

⁴ da Costa e Silva V. Policy recommendations for smoking cessation and treatment of tobacco dependence. Tools for Public Health. World Health Organization 2003. 107 pp. ISBN-13. 9789241562409.

⁵ Peto R, Lopez AD, Boreham J. et al. Mortality from smoking in developed countries, 2004. www.ctsu.ox.ac.uk New Zealand data.

⁶ Peto R, Lopez AD, Boreham J. et al. Mortality from smoking in developed countries, 2004. www.ctsu.ox.ac.uk

⁷ New Zealand Census March 2006. Smoking prevalence 20.7%. www.statistics.govt.nz

2. Description

The Ruyan® (pronounced Roo yen) (e for electronic) cigarette, like a tobacco cigarette, can rapidly deliver nicotine into the lungs, but without smoke carcinogens and toxicants.

The Ruyan® e-cigarette was first sold in May 2004, in China, with annual sales since of around 300,000 per year, and advertising on television, but no adverse effects reported by the English language dailies in China. Its December 2007 internet price was around US \$208, with nicotine cartridge refills required every 300 puffs (1-4 days) costing extra. After 1300 puffs the battery is recharged from the mains.

2.1 Structure.

The distal segment with a red light indicating inhalation, contains the re-chargeable battery and is the controlling part. The middle part contains a vaporising chamber. The mouthpiece and nicotine cartridge are one piece, and a new one is inserted after 300-350 puffs. The nicotine in the cartridge is dissolved in propylene glycol (PG). Table 2.1 enables estimation of the weight of the liquid in the container.

Table 2.1. Ruyan V8 electronic cigarette 16 mg cartridge, components by weight

Component		g	g	g
Battery (distal segment)				13.9
Atomiser (middle segment)				8.58
Cartridge (mouth end segment)				
Cartridge part (white)			1.67	
	Plastic shell	0.436		
	Silicon foam, dry	0.061		
	Liquid, full	1.173*		
Mouthpiece (black)			0.89	
				2.56

* Estimated by subtraction. Of the 1.173 g, 1g may be extractable.

2.2 Function.

The Ruyan® e-cigarette is flameless and non-flammable. The pressure sensor in the controlling part electronically initiates rapid vaporisation of a dose of liquid propylene glycol containing nicotine into a fine aerosol that reaches the lung rapidly.⁸ The dose per puff depends on the volume and force of the inhalation, and the number of puffs determines total dose.

3. Nicotine effects

The safety and toxicity of nicotine has been exhaustively reviewed.⁹ The safety of pure nicotine alone, relative to tobacco smoking is not in question, nor is its overall safety in

⁸ Hon, Lik. China. A non-smokable electronic spray cigarette. Patent CA 218174, published 2004/03/08.

⁹ Nicotine Safety and Toxicity. Ed. NL Benowitz. Oxford. OUP. 1998.

absolute terms. Death has been recorded occasionally from accidental poisoning from nicotine (Section 7), but not from medicinal use.

No nicotine poisoning effects have been reported for the Ruyan® e-cigarette. In contrast to the use of alcohol or oral snuff, the very rapid absorption enables the user to become aware of the first effects (light-headedness, queasiness) before serious overdosing can occur.

3.1 Short-term effects

Dose-control. For each puff, “what you inhale is what you get”. The smoker is protected from unwanted nicotine by the electronic circuitry shutting off almost immediately after each puff is taken. The smoker controls the size of the puff which determines the nicotine dose. The strength of the dose is immediately and correctly signalled by the irritation to the back of the throat, as no menthol is used to anaesthetise it. Thus the smoker is able to accurately control the dose from puff to puff.

With a zero-nicotine Ruyan® e-cigarette, there is no harshness on the throat, and without such negative feedback, the smoker may puff more frequently, but virtually no nicotine is inhaled. Purchasing 16 mg, 11 mg, 6 mg or 0 mg nicotine strengths of cartridge provides another way in which Ruyan® e-cigarette smokers can pre-regulate their nicotine intake.

Efficiency. No nicotine is wasted in the Ruyan® e-cigarette— over one to four days its nicotine is eventually all inhaled, thus differing from the 12% uptake of nicotine from the tobacco cigarette. In the tobacco cigarette, after combustion, most is lost in side-stream smoke. Of the mainstream smoke some is entrapped in the cigarette filter, while only 1.5 mg (12%) of the cigarette’s original nicotine content of 13 mg is inhaled. (Table 1).

In first-time smokers. Acute nicotine toxicity occurs when never-smokers smoke their cigarette (whether tobacco or e-cigarette), becoming light-headed, with nausea and even vomiting, lasting typically for half an hour. Many would-be smokers are thus discouraged from learning to smoke.

Maintenance of steady nicotine blood levels. The experienced smoker of the Ruyan® e-cigarette controls the nicotine intake to maximise pleasure and minimise discomfort. A regular e-cigarette or tobacco cigarette smoker adjusts the size or frequency of each subsequent puff, to maintain nicotine blood levels high enough to avoid unpleasant craving for a cigarette, and low enough to avoid excessive harshness on the back of the throat, or light-headedness due to a high blood level of nicotine.

Self-medication. In a relaxed situation, a smoker may deliberately inhale to achieve the nicotine rush or buzz or light-headed feeling, which will pass within half an hour or so. This is nicotine self-medication, or drug-effect seeking behaviour, which many smokers practice. Inhaling to the point of light-headedness can be harmful for tobacco smokers, tobacco snuff users and e-cigarette smokers who have to drive a car or operate heavy machinery immediately afterwards.

3.2. Long term effects. Thousands of smokers and former smokers have used nicotine in the form of gum for five years with no increase in mortality or hospitalisation.³

Longevity The cumulative excess risk of continuing to smoke tobacco cigarettes beyond age 35 years is one in two.² As the Ruyan® e-cigarette carries no risk to longevity, the average smoker switching to the Ruyan® e-cigarette before age 35 years will reduce their risk of dying early by one in two.

Cancer and Cardiovascular toxicity. Nicotine is not a cause of cancer. The tendency for nicotine to temporarily increases heart rate and blood pressure flattens out above 8 mg yield per cigarette, so that low doses produce much the same effect as high doses,⁶ suggesting that nicotine does not cause cardiovascular toxicity.

3.3 Previous tobacco smoking puts e-cigarette users at risk. Ruyan® e-cigarette users will be mainly current or past tobacco smokers, and for that reason are at increased risk of heart attack, stroke or lung cancer. Tobacco cigarette smokers have two to three times the annual death rate of non-smokers, and have ten times the risk of sudden cardiac death. Deaths of e-cigarette users may be wrongly blamed on their new e-cigarettes, rather than their past smoking of tobacco.

3.4 Dual use. Smokers may take some time to switch completely from tobacco to nicotine smoking. As long as they continue to smoke even a few cigarettes a day their risk of dying early remains excessive. (The risk of smoking even 1-4 cigarettes a day carries a 60% excess risk of dying early. Smoking 5-9 cigarettes a day doubles the risk of dying early, compared with never smoking²). In particular their excess risk of heart attack will not diminish substantially until they quit tobacco smoking entirely.

4. Nicotine dose, consumption, and labelling

4.1 Correct dose. Each smoker is accustomed to a certain amount of nicotine each day. This varies greatly between smokers, but for each smoker, varies little from day to day. Heavy tobacco cigarette smokers in the United States smoking an average 36 cigarettes (range 20-62) per day absorb about 37 mg per day (range 10-79 mg)¹⁰.

The Ruyan® e-cigarette cartridge can supply 16 mg nicotine per day. Non-inhalers and smokers of light cigarettes inhale less nicotine. If smoking one cartridge of the 16 mg Ruyan® e-cigarette per day is not able to control cravings, a second e-cartridge for the day might be needed.

Table 4.1 shows that, the 16 mg nicotine Ruyan® e-cigarette cartridge provides nicotine equal to 7 to 10 factory-made tobacco cigarettes. Once the smoker stops smoking tobacco cigarettes, the Ruyan® e-cigarette by itself is unlikely to cause nicotine overdose. Any smoker becoming light headed while smoking an e-cigarette, should stop smoking tobacco.

¹⁰ Benowitz *ibid.* p.6.

Table 4.1 Nicotine content and delivery or absorption per puff, per smoke, and per day, factory-made tobacco cigarette and Ruyan® e-cigarette compared.

	Content Nicotine in each unburnt tobacco cigarette, or in each Ruyan® e-cigarette cartridge** mg	Per puff Nicotine delivery per puff; 99% absorbed### mg	Per smoke Nicotine delivery and absorption# per cigarette or Ruyan® e-cigarette 'smoke' mg	Per day* Nicotine delivery and absorption (per 300 puffs from 20 cigarettes or 20 Ruyan® e-cigarette 'smokes' mg
	A	B**	C	D
Factory-made cigarettes	A	B=C/15	C	D=C*20
Regular filter cigarettes	13	0.16	1.4 to 2.4 assume 2.0	28 – 48, assume 38
Ruyan® e-Cigarettes	A	B=A/300***	C=b*15	D=C*20
Ruyan® cartridge Label: 16 mg	14~ - 16	0.053	0.80	14-16
Ruyan® cartridge Label = 11 mg	10~ - 11	0.037	0.56	10-11
Ruyan® cartridge Label 6 mg	6~	0.02	0.3	6
Ruyan® cartridge Label 0 mg	0-0.5~	0	0	0-0.5

* Ruyan® cartridge lasts one to four days. If it lasts four days, divide Ruyan values in D by 4.

** Assumes 15 puffs per cigarette.

*** Assumes 300 puffs per Ruyan® e-cigarette cartridge. Smokers taking larger puffs may finish the cartridge before 300 puffs.
~ Benchtop test value.

Nicotine absorbed per cigarette = 1.4 mg (Fagerstrom, for Sweden),¹¹ 2.4 mg (Djordjevic for USA).¹² The nicotine absorbed from tobacco smoking is much greater than what is printed on cigarette packets.

When nicotine aerosol is inhaled into lungs, approximately 99% of nicotine is retained.¹³

~ ESR Porirua October 2007.

4.2 Nicotine consumption per day. As puffs from the 16 mg nicotine Ruyan® cartridge contain one third to one half the nicotine in a tobacco cigarette puff, and Ruyan® e-cigarette smokers take up to four days to finish a cartridge, smokers are most unlikely to absorb more nicotine from Ruyan® e-cigarettes than previously absorbed from tobacco.

Smokers of the Ruyan® e-cigarette say a cartridge lasts 1 to 4 days, which for a 16 mg cartridge is equal to 4 to 16 mg per day daily or equal to 2.5 to 10 tobacco cigarettes a day unaccompanied by tar or gas toxicants (Table 1). Pure nicotine in this dose is neither excessive nor harmful.

As virtually all cartridge nicotine is eventually inhaled, and over 98% of inhaled nicotine is absorbed¹³ the consumption of nicotine cartridges per smoker will reliably establish the level of nicotine absorption per day, provided no tobacco or other nicotine product is being used. This enables clinicians and researchers to estimate nicotine consumption with more precision than is possible with tobacco consumption.

4.3 Nicotine per puff

Method 1) ‘Smoke’, 35 ml per puff, was drawn from the mouthpiece of the Ruyan e-cigarette until no more was obtainable. The total puffs per cartridge were thus documented for the manufacturer at a well-known Beijing laboratory.¹⁴

2) An e-cigarette (0 mg nicotine) was smoked for a total of 80 breaths – 40 shallow, and 40 by deep lung inhalation. The puffing was measured by CreSSmicro interposed between smokers and the e-cigarette. (Table 4.3)

Results.

- 1) By volume measurement, the manufacturer’s estimate was 350 breaths per cartridge. Table 4.1 is based on this estimate.
- 2) By weighing the decreases per ten puffs taken, (Table 4.3) the average weight of liquid used per puff was 1.5 mg for lung inhalations, and 0.5mg for shallow mouth-throat inhalation. As extractable propylene glycol is approximately 1.0 g, one cartridge should provide 667 lung inhalations.

Conclusion.

These two methods give different results. Further tests in human subjects will clarify how many puffs are obtained by most smokers per cartridge.

If the e-cigarette contained a 16 mg per 1 g of cartridge liquid, instead of the 0 mg cartridge as in Table 2, then, assuming the nicotine was equally concentrated across all puffs, and assuming all breaths are lung inhalations, 1.5 mg of liquid went into the average puff, providing an estimated $1.5 * 16\mu\text{g}$ nicotine per puff = 24 micrograms of nicotine. Fifteen puffs would thus supply 360 ug or 0.36 mg of nicotine, that is one fifth of the amount from one cigarette. And 15 shallow puffs would only supply 0.12 mg of nicotine. Volume and weight calculations thus give differing values. Pharmacokinetic testing will show whether smokers obtain sufficient nicotine from the e-cigarette.

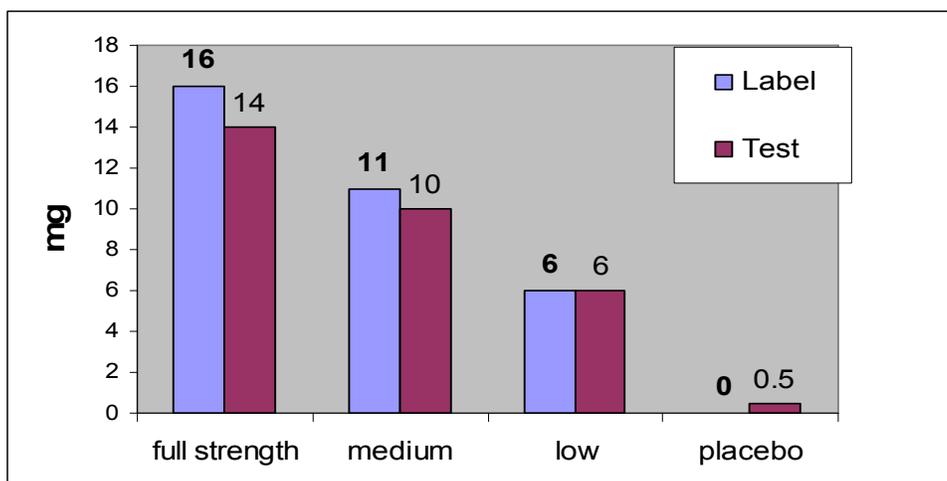
Table 4.3. Weight loss of the 0 mg e-cigarette as a measure of vapourisation of the cartridge liquid

Method	Average puff duration in seconds	Average volume per puff mL	Puff Count	Weight change in e-cigarette Mg	Weight loss per breath mg
Inhaled into mouth and throat (puff volume 21 ml)					
Mouth/throat	0.76	21.1	40	20	0.5
Inhaled into lungs (average puff volume 44 ml)					
Total lung	1.63	43.8	40	60	1.5
Total all	1.20	31.9	80	80	1.0

Lung inhalation results in three times as much cartridge liquid being inhaled, as from shallow inhalation confined to mouth and throat.

4.4 Accuracy of nicotine dose labels

Table 4.4 Ruyan® e-cigarette per cartridge nicotine content by label and by test



The only biologically active ingredient expected in the Ruyan® e-cigarette cartridge liquid is nicotine. On analysis¹⁵, the cartridges labelled as 16 mg actually contained 14.1 mg of nicotine; those labelled 11 mg contained 10.0 mg; those labelled 6 mg contained 5.9 mg; and those labelled 0 mg of nicotine contained 0.5 mg.

In a separate estimation, the nicotine alkaloid β -nicotyrine ($C_{10}H_{10}N_2$) was detected on scan of the headspace in the cartridge by microextraction. The area under the peak was 4% that of nicotine. ($C_{10}H_{12}N_2$). This requires re-analysis once the source of nicotine for the e-cartridge is upgraded.

5. Risk of addiction

5.1 Tobacco versus nicotine addiction.

The extent of the addictive potential of the Ruyan® e-cigarette is not yet known. The frequency of nicotine addiction is lower for all nicotine products so far developed, than for tobacco products, but the e-cigarette represents the first time a pure-nicotine-smoking product is available to be compared with a tobacco cigarette. The illness consequences of addiction to lit tobacco smoke however, are infinitely greater than from addiction to nicotine; without the products of combustion.³

Other active substances in tobacco. MAO inhibitor compounds (such as harman and norharman) in tobacco smoke are believed by many to potentiate the effect of the nicotine. If this proves to be a strong factor with respect to tobacco smoke, then the nicotine-only e-cigarette will be much less addictive than smoking tobacco cigarettes. Vapours from the e-cigarette cartridge do not inhibit MAO enzymes. See 8.1.2

Other factors. The cost of buying the e-cigarette, and the need to use a credit card to order it by mail, will likely deter most young people obtaining the e-cigarette for personal

use. If some youths do use it, and develop a taste for nicotine, the price of nicotine refills versus cigarettes will be decisive for many. It also depends on fashion, safety concerns, and whether parents, health groups and doctors approve its use.

5.2 Addiction in smokers

The Ruyan® e-cigarette does not cause nicotine addiction in smokers, as most cigarette smokers are already addicted to nicotine. E-cigarettes are not expected to increase the need for nicotine in smokers, as each smoker needs a certain amount of nicotine each day, and the brain receptor cells cannot distinguish where the nicotine molecule comes from (smoked tobacco, tobacco snuff, or e-cigarette). The e-cigarette does not increase the daily customary dose; from the first day of using the Ruyan® e-cigarette most smokers tend to smoke very few tobacco cigarettes, and surprisingly, seem to use few e-cigarette cartridges in their place. This however is yet to be researched.

Once the daily dose is obtained, the smoker will not reach for another puff from either their tobacco cigarette or their e-cigarette: no pleasure is obtained and there is no craving to relieve.

Further research on quitters is required to find out how many will prefer to continue using the e-cigarette one year after quitting smoking.

Smokers quitting smoking in countries which encourage quitting, are likely to use the e-cigarette to gain control of their nicotine needs, and use it temporarily – for a few weeks only, after stopping smoking. Quitting smoking is often part of a lifestyle change which will often include quitting tobacco and nicotine altogether. By the time smokers are ready to quit cigarettes, many also want to end their nicotine addiction.

The 16 mg, 11 mg nicotine cartridges are expected to satisfy the cravings and maintain the addiction of smokers who wish to stay on nicotine. This is the subject of further tests in 2008.

On the basis of similarity to the rapid action of nicotine nasal spray, we assume that one year after smokers' first using the Ruyan® e-cigarette as a stop smoking aid, no less than 15% would become long term users. (See data for nicotine nasal spray below)..

The 6 mg nicotine cartridge if used up in one day may provide just enough nicotine to maintain addiction. Used over 4 days it would not be sufficient. Very recent quitters using the 6 mg cartridge would likely have cravings for cigarettes and be at risk of smoking tobacco again.

The 0 mg nicotine-labelled cartridge will not maintain addiction. It will provide 0.002 mg nicotine per puff, 0.025 mg per smoke, which at even 300 puffs a day amounts at most to 0.6 mg per day, much less than the estimated 5 mg daily required to sustain addiction¹⁶. The labels were therefore safe estimates of the dose of nicotine to be expected, and the 0 mg e-cigarette can be used without risk of creating or maintaining dependence on nicotine. Nicotine is not recommended for non-smokers but for smokers already addicted (dependent) to nicotine, who wish to avoid inhaling tobacco smoke.

Tobacco cigarettes Some 84% view their own use of cigarettes as an addiction¹⁷.

Comparison with nicotine medications (Nicotine Replacement Therapy, NRT) Of users of nicotine medications some are still using the medication after one year and assumed to be addicted. (2% for patch, 8% for spray, 9% for gum and 15% for nasal spray)¹⁸.

6. Addiction in young people

Tobacco, tobacco snuff, nicotine snuff (Niconovum) and the e-cigarette can all be expected to induce nicotine addiction in many young people. This involves a subtle loss of autonomy or control over their new habit. Once addicted to nicotine, the concern is that tobacco, snuff and the e-cigarette could be used interchangeably.

The answers to this concern depend on what policies society has put in place to steer young people away from addiction of any kind, and away from tobacco smoking in particular. In 2007, New Zealand smokers could buy an e-cigarette from the internet for the price of a carton of cigarettes. If cigarettes cost much more, more would buy the e-cigarette. Similarly, graphic disease warnings on cigarette packets may persuade smokers to quit or switch to other alternatives such as the e-cigarette which do not require or merit such disease warnings.

Addiction to smoking tobacco cigarettes ensures young smokers remain smokers into adult life and continue to smoke beyond 35 years of age when the risks of smoking deaths begin to increase. Similarly smoking the e-cigarette makes it less likely that the smoker will ever want to smoke tobacco cigarettes again.

Young people may use the e-cigarette as a temporary crutch while stopping smoking and so avoid the future increased mortality risks of smoking. If the e-cigarette was widely available to young people, their cigarette smoking would decrease, life expectancy increase and respiratory health would improve, without any extra mortality from nicotine.

The fate of users of the e-cigarette

Smokers who try the e-cigarette will either:

- Try the e-cigarette experimentally, then revert to tobacco smoking as before.
- Use the e-cigarette as a temporary aid to quitting smoking entirely.
- Switch permanently to e-cigarette (and no longer smoke tobacco) .
- Continue to use both e-cigarette and tobacco cigarettes (See 3.4 above).

First cigarettes The first tobacco cigarettes smoked result in one on four adolescents losing some autonomy (control) over their smoking¹⁹. Whether some adolescents would soon lose partial control over their use of the Ruyan nicotine e-cigarette is not clear.

Addictive potential The proportion of young people who will prefer the e-cigarette over tobacco, and who use it long term, is unknown. It will vary by country. If government and health groups regard the e-cigarette as almost as dangerous as smoking, e-cigarette

smokers may adopt a “why not” approach to tobacco cigarette smoking, and tobacco cigarette smoking will not be reduced. If, however, society approves the e-cigarette as a cigarette alternative, its users can smoke it openly, without damaging their health. It will be a much safer habit than either smoking tobacco or drinking alcohol.

Graphic and varied health warnings The e-cigarette is not likely to cause addiction any more than cigarettes, which most adolescents can obtain with ease. On the other hand, the e-cigarette supplies safe nicotine, without risk of early death due to lung cancer, heart disease, or emphysema. A simple, truthful warning is therefore suggested below for the e-cigarette.

Graphic warnings on cigarette packets will discourage tobacco smoking.. Better information about the health risks of smoking, and higher prices for tobacco, will mean that as the e-cigarette becomes available, the proportion of young people smoking tobacco should decrease more rapidly.

Possible health warning

This nicotine product is addictive but avoids the other risks of smoking.

Tobacco cigarettes in many countries now warn the smoker “Smoking is addictive”. Similar warnings are needed on the e-cigarette packaging, pointing up the difference between the e-cigarette and tobacco cigarettes. Although the manufacturer’s pamphlet warns that the e-cigarette is not suitable for young people or non-smoking adults, some may gain access to it.

If young people see graphic disease warnings and high prices on tobacco packets, young people will abstain from tobacco smoking or possibly switch to e-cigarette smoking instead. In due course, fewer will die early from tobacco smoking.

Conclusion.

The invention of the e-cigarette means society must now distinguish between

- Very harmful (tobacco) smoking, and harmless (e-cigarette) smoking; and
- very harmful addiction (associated with smoking) and fairly harmless addiction (associated with the e-cigarette).

Regulators in Western countries are likely to

- prohibit sale of nicotine e-cigarette refills to under-18s, in line with restrictions on cigarette sales to youth
- permit e-cigarette use in most areas where tobacco cigarette smoking is banned
- permit e-cigarette advertising in countries permitting advertising of NRT.
- continue to enforce existing bans on the advertising of smoking tobacco.

7. Risk of accidental ingestion of nicotine

Accidental ingestion of the e-cigarette is theoretically possible, though it has not been mentioned in the English news media in their articles on the Ruyan® e-cigarette from China, where 300,000 units are sold annually since 2004. Poisoning by ingesting tobacco cigarettes is rare, even though children can easily access tobacco cigarettes in the home.

7.1 Ruyan nicotine cartridges, when sold separately are packed in individually sealed child-proof canisters. Without scissors, even adults find them difficult to open. In this packaging, unattended children in car or home are not at risk from the nicotine.

7.2 The nicotine cartridge assembled into the e-cigarette is better child-proofed than a packet of tobacco cigarettes.

- Many Ruyan® smokers keep their e-cigarette close by, reducing risk of child access.
- Once assembled, the join between the e-cigarette mouthpiece/ cartridge, and the metal shell of the middle section is normally difficult even for an adult to pull apart. It is not a screw join.
- If, unusually, this join was loose, it prevents normal use of the e-cigarette, so does not remain loose for long.
- If unusually a child gained access to it and pulled it apart, put the cartridge in the mouth and sucked on it, then the nicotine impregnated in the cartridge could be absorbed through contact with mouth mucosa causing acute toxicity.

Swallowing is less likely, as the mouthpiece-cartridge measures 5 cm in length by 1cm diameter, and requires adult force to separate its two parts.

The highest dose e-cigarette cartridge contains 16 mg of nicotine. The factory-made tobacco cigarette contains 13 mg²⁰. The lethal nicotine dose for a child is known to be 10 mg.

8. Safety of the cartridge liquid and inhaled aerosol

Propylene glycol makes up 89-90% of the liquid in the nicotine cartridge that generates the aerosol inhaled by the e-cigarette smoke. (See Appendix 1, Table 2).

Propylene glycol is virtually non-toxic, See Appendix 3.

8.1 Tobacco flavour, Nitrosamines and MAO inhibitor effects

In cartridges dated November and December 2007, the fragrance, odour and taste of tobacco remained. The manufacturer's recipe (Appendix 1) suggests this comes from a flavour base containing tobacco extract weighing 6 mg per cartridge. As we now show, the cartridge liquid does not behave like tobacco:

8.1.1 Tobacco-specific nitrosamines Traces of these nitrosamines, found only in tobacco, were not found in the Ruyan® e-cigarette cartridge liquid except at trace quantity, at a level equal to that reported for medicinal Nicorette gum, at a low level uncharacteristic of tobacco. On this basis, the Ruyan® e-cigarette is a nicotine product, not a tobacco.

The maximum level of tobacco specific nitrosamines of 8 ng TSNA's per gram of cartridge liquid (8 parts per billion or ppb) found in 16 mg nicotine cartridges, compares closely to the 8 ng per gram found in Nicorette gum sold as a nicotine replacement therapy medicine in the United Kingdom²¹. The e-cigarette TSNA content is 200 times less than the amount found in Swedish moist snuff (1000 to 2400 ppb), and 150 times

less than the amount found in unburnt tobacco in the most popular filter cigarette and cigarette tobacco brands (1230 ppb)²².

Table 8.1 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 ²³ .					Average TSNAs	3.928
BDL = Below the limit of detection. NQ = Not quantifiable. TSNA = tobacco specific nitrosamines. NAB= nitrosoanabasine NNN= nitrosonornicotine, NAT= nitrosoanatabine, NNK= 4-nitrosomethylamino-1-(3-pyridyl)-1-butanone						

8.1.2 Monoamine oxidase. The cartridge liquid retains a tobacco fragrance or odour. (Appendix 1). Monoamine oxidase (MAO) enzymes both A and B, are strongly inhibited by tobacco smoke extract but the cartridge liquid had no such effect²⁴. MAO, found in blood platelets and the brain, has been regarded as a potentiator of the reinforcing (addictive) effects of nicotine. The test results have several implications:

- 1) The e-cigarette liquid in the cartridge lacks the MAO inhibiting effect of tobacco.
- 2) Any addictive potential of the e-cigarette is due to nicotine (complemented by nicotine analogues) but the nicotine effect is not reinforced by MAO. This provides a biomedical basis for the e-cigarette to be less addictive than smoking tobacco.
- 3) If the e-cigarette proves to be no less addictive than the tobacco cigarette, the difference could be explained by less nicotine inhaled from the e-cigarette; or it could be due to MAO in tobacco.
- 4) The closer the addictive potential of the e-cigarette to that of tobacco cigarettes, the less likely it is that MAO in tobacco is important in reinforcing nicotine's addiction potential.

8.1.3 Benzo(alpha)pyrene The cartridge liquid was tested for benzo(alpha)pyrene, a probable human carcinogen (detectable in tobacco cigarette smoke at 35 nanograms (ng) per cigarette). The value obtained from the e-cigarette liquid was below the method's limit of detection of 1 ng²⁵. As the e-cigarette cartridge is equivalent in nicotine to no more than 10 cigarettes, e-cigarette smoking delivers 350 times less benzo(alpha)pyrene than does tobacco cigarette smoking.

8.2 Volatile organic compounds (VOCs)

8.2.1 In e-cigarette ‘smoke’.

The yield of VOCs in the “smoke” of the e-cigarette has not been analysed. Volatile organic compounds are small molecules, the products of combustion, and found in all tobacco smoke. The e-cigarette generates no flame or fire; and does not heat up the e-cigarette. The very high temperatures of a burning cigarette (combustion) are not achieved in e-cigarette smoke.

Analysis of VOCs is planned before the next and final version of this report is issued.

8.2.2 In the e-cigarette cartridge liquid.

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

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

Method Ruyan e-Cigarette cartridges (16 mg nicotine; batch 20071228) had their wisp removed and one 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 analysed for comparison. Bottles were then incubated at 37 °C for approximately 60 minutes prior to analysis.

Method

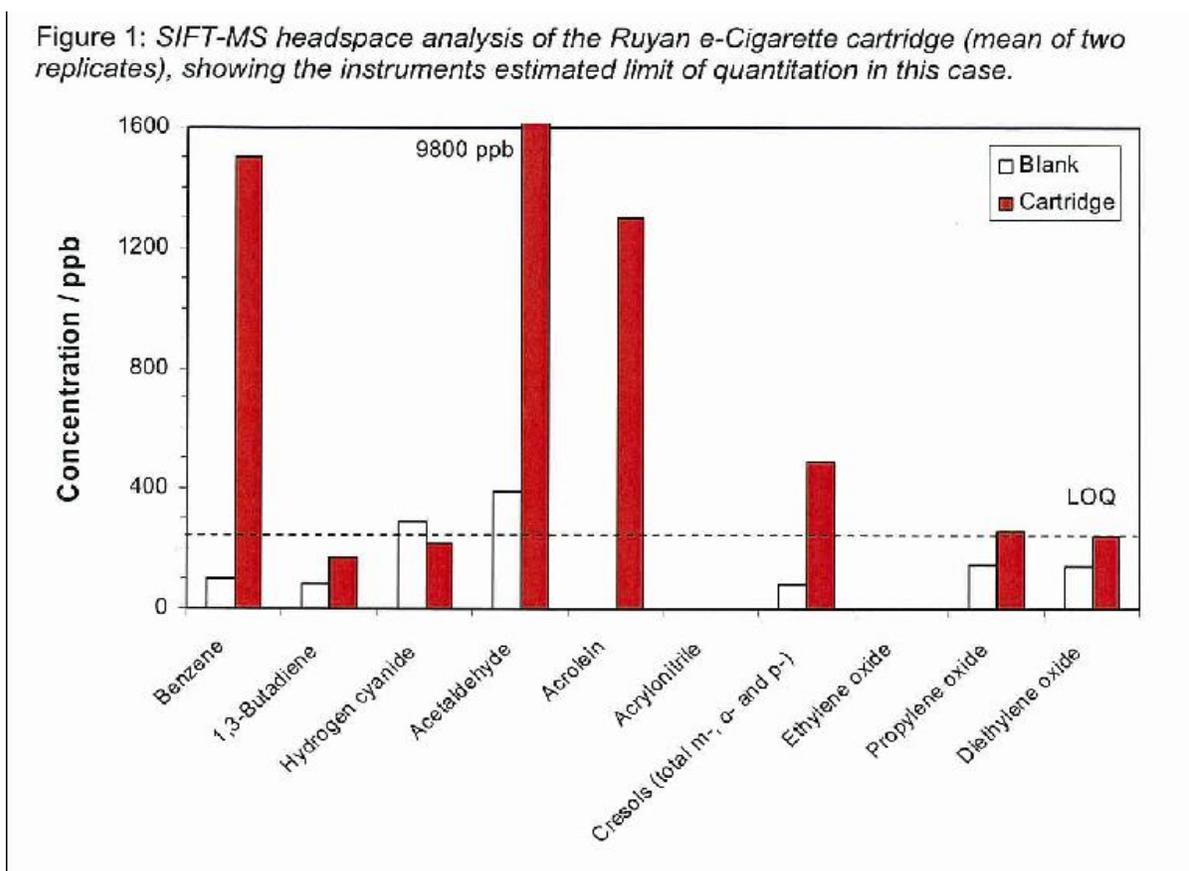
SIFT-MS analyses gas samples for volatile organic compounds (VOCs) and certain inorganic compounds.¹ Typically it can accurately detect and quantify these compounds in real time at very low concentrations (usually to parts-per-trillion {ppt} levels), even at breath humidity. SIFT-MS does not employ chromatographic separation and hence cannot perform well when high levels of organic solvents are present. A Syft Technologies Voice100™ instrument was used for this work. It was operated in two modes- selected ion mode (SIM) or Full Scan Mode (FSM).

Results

The results of the analysis are shown in Figure 8.2.2a and Table 8.2.2b. 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 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.

- Using SIFT-MS, (Figure 8.2.2a), due to interference from alcohol in the Ruyan cartridge, ethylene oxide could not be separated from acetaldehyde. Meantime, using HS SPME method (below) ethylene oxide was not detected in the headspace of the Ruyan cartridge, and therefore the 9500 ppb seen in Figure 1 is all due to acetaldehyde.
- Acrylonitrile does not register in the graph: no response was obtained. Although below the level of quantitation of 0.3 ppm, it suggests that acrylonitrile is absent.

Figure 8.2.2a: SIFT-MS headspace analysis of the Ruyan® e-cigarette cartridge (mean of two replicates), showing the instrument's estimated limit of quantitation.



“The results presented here are preliminary, due to interferences caused by ethanol, which is present at very high concentrations in the wick. The results represent an upper limit. However the measurements do show definitively that a number of tobacco-related toxicants are not present at significant levels in the e-Cigarette, such as hydrogen cyanide, 1,3-butadiene and acrylonitrile. For toxicants that appear to have concentrations above the limit of quantitation (LOQ), it is recommended that other techniques (for example, GC-MS or LC-MS) be used for more definitive analysis.”

Accordingly, on this recommendation we used GC-MS analysis (See 8.2.2B below).

Table 8.2.2b: SIFT-MS headspace analysis of the Ruyan® e-cigarette cartridge (mean of two replicates). “<LOQ” = less than the limit of quantitation.

Toxicant	Concentration in blank (parts per billion; ppb)	Concentration in headspace of cartridge (parts per billion; ppb)
Acetaldehyde	<LOQ	9500
Benzene	<LOQ	1500
1,3-Butadiene	<LOQ	<LOQ
Hydrogen cyanide	<LOQ	<LOQ
Acrolein	<LOQ	1300
Acrylonitrile	<LOQ	<LOQ
Cresols (total m-, o- and p-)	<LOQ	490
Propylene oxide	<LOQ	<LOQ
Diethylene oxide	<LOQ	<LOQ

Langford 2008²⁶

B) Volatile Profile of the Sample Ruyan® e-cigarette by using HS-SPME and GC-MS²⁷

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 fibre into the headspace of a sealed vial and allowing the volatile compounds in the headspace to absorb onto the fibre surfaces. These volatile compounds were then introduced into the GCMS by exposing the fibre 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 using this technique. 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 analysed 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).

Sample preparation involved placing the ecigarette 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 fibre (Supelco). During this exposure period the headspace volatiles were absorbed onto the fibre.

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).

Results

Table 8.2.2c. Screening of headspace vapour of a just-opened Ruyan® e-cigarette cartridge by different methods- SIFT-MS (Selected Ion Flow Tube with Mass Spectrometry), Solid phase microextraction (HS-SPME), and exhaled CO.

Compound	Toxicology *	MRLs Minimal Risk Levels non-cancer effects) ppm**#	PELs Permissible Exposure Levels of OSHA) ppm***	Detected in headspace vapour of Ruyan® e-cigarette cartridge Detected YES or NO	
				SIFT-MS Mass Screen ppm, 37deg C	HS-SPME 37deg C
Acetaldehyde	CA?, R.	Not listed	200	YES <9.5ppm	YES
Acetone	N	13 Chronic	1000	Not tested	YES
Acrolein	R	0.00004 I	0.1	YES < 1.3	NO
Acrylonitrile	CA, R	0.1 Acute	Not listed	NO. <<0.3	YES
Benzene	CA, CVD	0.003 Chronic	10	YES < 1.5	NO
1,3, Butadiene	CA	Not listed	1-5	NO <0.3	NO.
m-, o-, p- Cresols	CVD	Not listed	5	YES <0.495	NO
Carbon monoxide	CVD^	Exhaled.breath	50	15 puffs do not raise CO	
Ethylene oxide	CA	0.09 ppm I	Not listed	Not reported.	NO
Hydrogen cyanide	CVD	Not listed	10	NO < 0.3	NO
Propylene glycol	Not toxic	0.009 ppm I	None listed	YES	YES
Styrene	? CA	0.2 chronic	100	Not tested	YES
Xylenes	N	0.05 chronic	100	Not tested	YES

* CA= carcinogen, CVD= cardiovascular toxicant, N= neurological toxicant, R= respiratory toxicant.

^CVD risk for increased risk of ventricular fibrillation begins at 33 ppm (COHb=5%) and above.

<http://www.atsdr.cdc.gov/interactionprofiles/IP-12/ip12-a.pdf>

**Minimum Risk Levels for hazardous substances.US Public Health Service, Agency for Toxic Substances and Disease Registry. Nov. 2007. <http://www.atsdr.cdc.gov/toxpro2.html>

***Permissible Exposure Levels. OSHA. Sept. 2007.

http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992

Acute effect = 1-14 days, I=Intermediate effects = 14-364 days, Chronic effect = 365 days or longer.

< LOQ= below the limit of quantitation. ppm = at 37 degrees Centigrade.

Summarising Table 8.2.2c above, the volatile organic compounds detected had either

- High permitted level for chronic exposure, as for acetone, acetaldehyde, styrene or xylene. (10 to 1000 ppm), but not yet quantified, or
- No listing under OSHA, but not exceeding 0.3 ppm on SIFT-MS, or
- No listing under OSHA, lack of proven toxicity. Example: propylene glycol, Present in ample quantity.

Table 8.2.2d. Toxicants ranked by Permitted Exposure Levels,* and whether present in Ruyan® e-cigarette cartridge vapour as detected by HS-SPME and exhaled CO.

Permitted Exposure Level PEL, ppm*	Compound Ppm	Detection by HS-SPME	SIFT-MS	CO Monitor, Exhaled breath	Remarks
< 1 ppm	Acrolein	NO	1.3 ppm		Need to quantify further
10 or less	HCN, butadiene	NO	<LOQ		Major toxicants
50	Carbon monoxide			NO, Not increased	
100	Styrene	YES	9.5 ppm		OSHA permits higher levels in air for chronic exposure to these gases
100	Xylene	YES			
200	Acetaldehyde	YES			
1000	Acetone	YES			
Not listed by OSHA	Propylene glycol	YES	YES		Not considered toxic
	Acrylonitrile	YES	<<0.3 ppm, virtually zero.		Need recheck and quantify by a GC-MS method
	Ethylene oxide	NO			

*Permissible Exposure Levels. OSHA. Sept. 2007 for weighted average exposures during an 8 hour shift.

http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992

For a graphic of the run result, see www.healthnz.co.nz/Portland2008ECIG.pdf

8.2.3 Analysis of the exhaled breath after using the Ruyan® e-cigarette

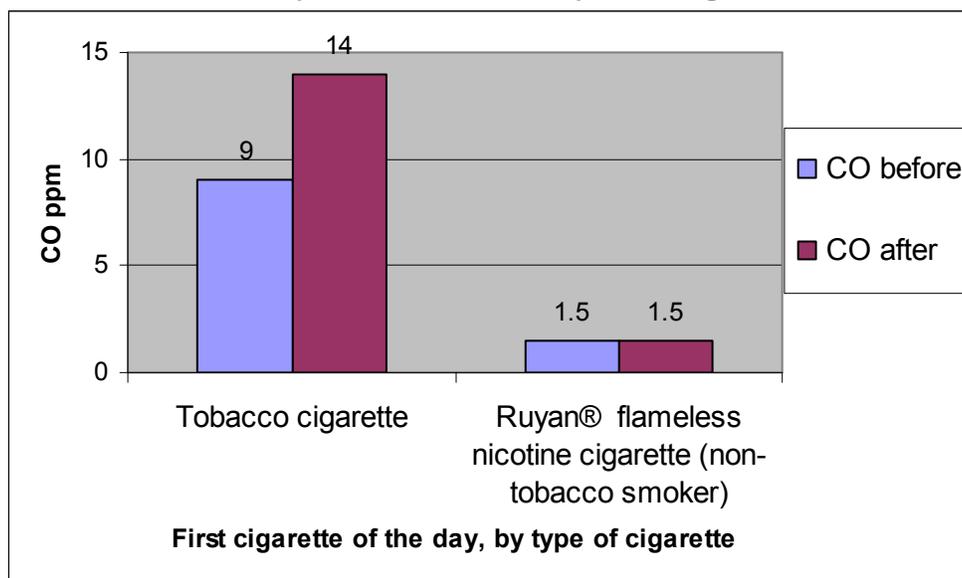
Carbon monoxide is a product of combustion and therefore can distinguish between the smoke produced by burning tobacco versus flameless cigarettes.

Method

Five minutes after their final puff of their first cigarette of the day, 17 smokers exhaled into a MicroMedical CO analyzer.²⁸ A non-tobacco smoker with a smokefree home and workplace, was similarly tested after 20 lung inhalations from of the Ruyan® e-cigarette.

Results

Table 8.2.3 Carbon monoxide in exhaled breath, before and after the first cigarette of the day, tobacco versus Ruyan® e-cigarette



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

8.3 Impurities

8.3.1 In Propylene glycol. Impurities might arise in the manufacture or storage of propylene glycol.

Propylene oxide and ethylene oxide (a carcinogen) were not detected above the limit of detection 16.75 ug/ml and 42.5 ug/ml respectively on GCMS (gas chromatograph, mass spectrograph) testing.²⁹ Some interference (matrix effect) prevented accurate quantification. However neither compound was detected by the HP-SPME scan, suggesting their levels if present were likely to be under 1 ppm.

8.3.2 Heavy metal traces. Heavy metals such as chromium, arsenic, and nickel can cause cancer, and lead is a neuro-toxicant. The liquid was tested for heavy metals (Arsenic, Antimony, Cadmium, Chromium, Cobalt, Copper, Lead, Manganese and Nickel), and the concentrations in each case were less than 1 part per million. No hazardous effects are expected from heavy metals at this concentration².

9. Risk of cross-infection from use

9.1 Risk of contamination from the mouthpiece. Public health agencies typically advise smokers not to share drinking glasses or cigarettes, due to the risk of cross-infection from lip saliva on the mouth end, with the risk of meningitis. This advice holds true for any electronic cigarette.

9.2 Risk of micro-organisms in the cartridge liquid. Another risk would be if the liquid in the cartridge acted as a culture medium for micro-organisms. The 5% alcohol content of the cartridge liquid (See Appendix 1) might be expected to inhibit growth of micro-organisms.

Environmental Science Research tested one used and one unused Ruyan® cartridge for the presence of the three main classes of micro-organism (aerobic, anaerobic and Legionella)³⁰. None was found.

We conclude there is no inherent tendency in the design of the Ruyan® e-cigarette towards contamination from growth of organisms in the cartridge liquid. Nevertheless, instructions to users (and to tobacco cigarette smokers) should discourage cigarette sharing because of the risk of transfer of meningococcal meningitis, tuberculosis and other infectious diseases.

10. Safety of Ruyan® e-cigarette ‘smoke’ for bystanders.

Because inhaled nicotine is over 98% absorbed⁶, the exhaled ‘smoke’ is propylene glycol minus the nicotine, and any exhaled PG mist dissipates within seconds. Without the gaseous products of combustion, the ‘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. and e-cigarette “smoking” would be permitted under New Zealand’s Smoke free Environments Act³¹.

11. Further safety testing

Analyses have been requisitioned for further testing for possible impurities in the cartridge liquid.

Also, in January to March 2008, as part of a further trial of safety and efficacy, Clinical Trials Research Unit, University of Auckland independently monitored the use of the e-cigarette by some 50 subjects, over the course of one day, and recorded any adverse effects. These results are not yet available, and so will form the basis of a separate report to be published later in 2008.

Appendix 1. Safety 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%
Flavour base *	0.6%	0.6%	0.6%	0.6%
Total	100%	100%	100%	100%

Source: Table 1.1.

*** See Appendix 3. Safety of 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) is found in beef, coffee, popcorn, sesame seed, almond, wheat bread, cocoa, peanut, pork and potato chips, etc. According to the documentation from tobacco industry, acetyl pyrazine is added to cigarettes to give a pop-corn-like flavor and aroma to the tobacco.

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

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Appendix 2. Ruyan® e-cigarette. New Zealand testing to date, as of 9 April 2008

Topic	Name of test	Purpose	Status	Result
Toxicology	Nicotine content of liquid in cartridges	Confirm labelling states contents correctly	Completed	Generally around 90% of label.
	Benzo-alpha-pyrene in liquid	Whether liquid carcinogenic	Completed	None found
	Heavy metal traces in cartridge liquid	Whether liquid carcinogenic	Completed	Less than one part per million
	Tobacco specific nitrosamines in cartridge liquid	Whether liquid carcinogenic	Completed	Same as in Nicorette gum
	MAO inhibitors found in tobacco	Whether tobacco like effect found.	Completed	MAO effect not detected.
	Headspace tests for volatile organic compounds	To detect any impurities in cartridge liquid	Completed	Some detected and need quantifying.
	Draw-over 'smoke' tests	for quantifying gases detected	Booked for April 2008	Available May 2008
	Test for bacteria	To rule out infectivity. Whether bacteria grow in used and unused cartridges.	Completed	No growth of aerobic, anaerobic bacteria or legionella
Adverse effects	50 smokers to use each product for one day	Note how adverse events compare.	February-March 2008	Expected May 2008
Satisfaction ratings	50 smokers to use 16 mg Ruyan® e-cigarette for one day; and on other days use 0 mg Ruyan® e-cigarette, 10 mg Nicorette inhaler and own cigarette.	Rate satisfaction with product at end of day.	February-March 2008	Expected May 2008
Efficacy Effect on urge to smoke (cigarette cravings)		Compare urge to smoke before are many times after using each product.	February-March 2008	Expected May 2008
Efficacy Pharmacokinetic study	Blood nicotine taken before and after using Ruyan® e-cigarette. (12 tobacco smokers)	Test and compare increase in blood nicotine after use of each product over two hours.	February-March 2008	Expected May 2008

Appendix 3. Safety of Propylene Glycol

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

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.²

Animal studies

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.³

Addition of propylene glycol at 2.2% w/w tobacco does not increase the toxicity of cigarette tobacco.⁴ In rats PG levels in plasma and lung are super-imposable with half an hour. A mild cumulative build up (30% or less) occurred after 28 days.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 vapour has 100% deposition efficiency in human airways.⁷

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

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.

Toxicity The website www.pneumotox.com devoted to inhalational toxicology, registers one case report of bronchospasm⁸ but no other adverse effects.

Since PG is less efficiently absorbed following dermal and inhalation exposure compared to oral exposure, it is likely to have a low acute toxicity by these routes of exposure. CNS depression causing mortality has been described in premature infants after repeated exposure to medication containing PG.⁹

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.

Absorption PG is absorbed rapidly and completely when taken orally. Humans have been given 40 g per 12 hours for 3 days to establish a steady state. After 3 days blood levels reached maximum one hour after administration of the PG dose.² We could find no data on the proportion of PG absorbed by inhalation. However the proportion is expected to be high, as it is highly soluble.

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.² This NOEL, however, is artificially low - an artefact of the vapour 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.¹⁰

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

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