FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

**6-04**
**4 August 2004**

# FINAL ASSESSMENT REPORT

# PROPOSAL P278

# USE OF NICOTINE AND *NICOTIANA* SPECIES IN FOOD

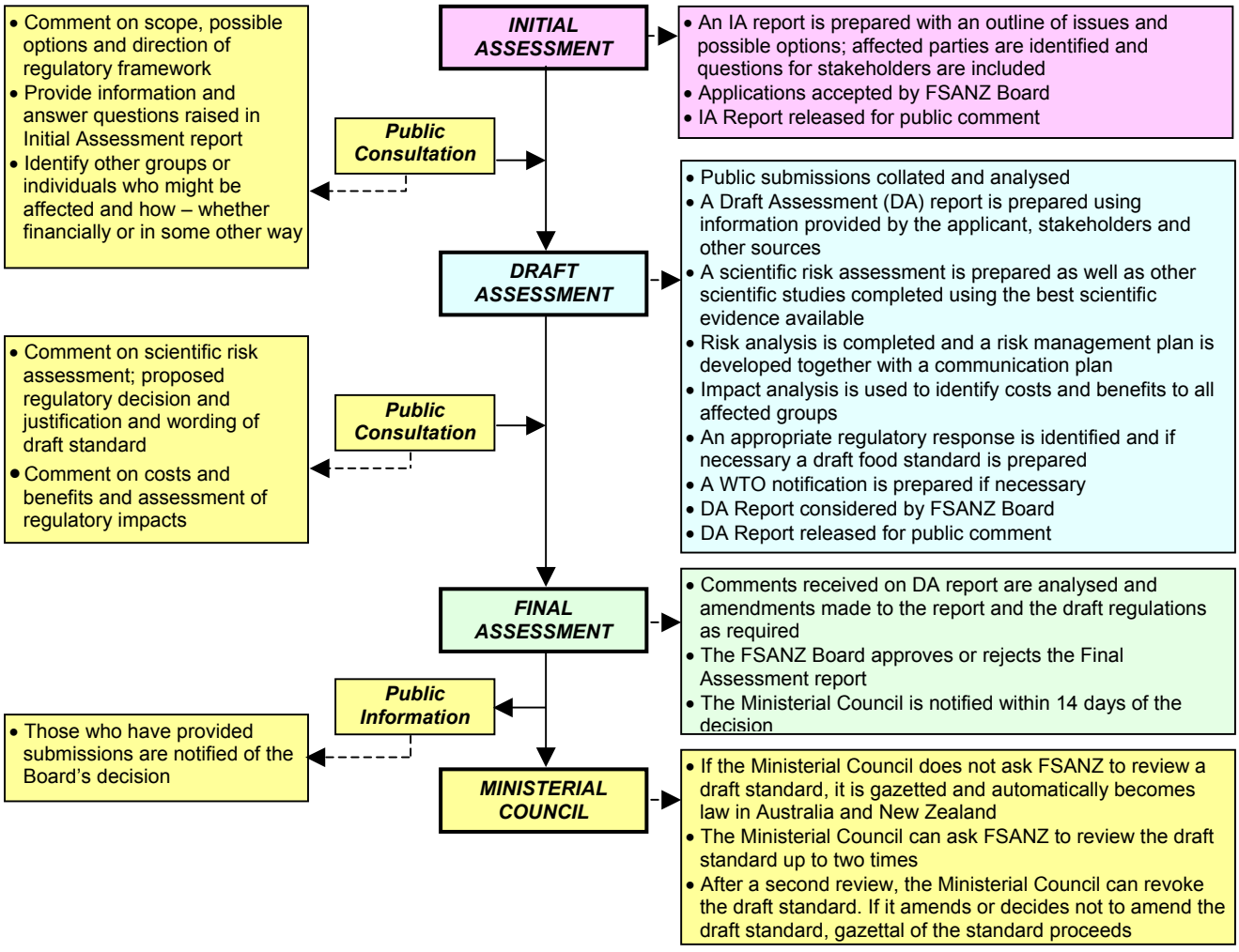
## FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

FSANZ's role is to protect the health and safety of people in Australia and New Zealand through the maintenance of a safe food supply. FSANZ is a partnership between ten Governments: the Australian; States and Territories; and New Zealand. It is a statutory authority under Australian law and is an independent, expert body.

FSANZ is responsible for developing, varying and reviewing standards and for developing codes of conduct with industry for food available in Australia and New Zealand covering labelling, composition and contaminants. In Australia, FSANZ also develops food standards for food safety, maximum residue limits, primary production and processing and a range of other functions including the coordination of national food surveillance and recall systems, conducting research and assessing policies about imported food.

The FSANZ Board approves new standards or variations to food standards in accordance with policy guidelines set by the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) made up of Australian, State and Territory and New Zealand Health Ministers as lead Ministers, with representation from other portfolios. Approved standards are then notified to the Ministerial Council. The Ministerial Council may then request that FSANZ review a proposed or existing standard. If the Ministerial Council does not request that FSANZ review the draft standard, or amends a draft standard, the standard is adopted by reference under the food laws of Australia, States, Territories and New Zealand. The Ministerial Council can, independently of a notification from FSANZ, request that FSANZ review a standard.

The process for amending the *Australia New Zealand Food Standards Code* is prescribed in the *Food Standards Australia New Zealand Act 1991* (FSANZ Act). The diagram below represents the different stages in the process including when periods of public consultation occur. This process varies for matters that are urgent or minor in significance or complexity.



**Final Assessment Stage**

FSANZ has now completed two stages of the assessment process and held two rounds of public consultation as part of its assessment of this Proposal. This Final Assessment Report and its recommendations have been approved by the FSANZ Board and notified to the Ministerial Council.

If the Ministerial Council does not request FSANZ to review the draft amendments to the Code, an amendment to the Code is published in the *Commonwealth Gazette* and the *New Zealand Gazette* and adopted by reference and without amendment under Australian State and Territory food law.

In New Zealand, the New Zealand Minister of Health gazettes the food standard under the New Zealand Food Act. Following gazettal, the standard takes effect 28 days later.

**Further Information**

Further information on this Proposal and the assessment process should be addressed to the FSANZ Standards Management Officer at one of the following addresses:

**Food Standards Australia New Zealand**
**PO Box 7186**
**Canberra BC ACT 2610**
**AUSTRALIA**
**Tel (02) 6271 2222**
**www.foodstandards.gov.au**

**Food Standards Australia New Zealand**
**PO Box 10559**
**The Terrace WELLINGTON 6036**
**NEW ZEALAND**
**Tel (04) 473 9942**
**www.foodstandards.govt.nz**

Assessment reports are available for viewing and downloading from the FSANZ website www.foodstandards.gov.au or alternatively paper copies of reports can be requested from FSANZ's Information Officer at info@foodstandards.gov.au including other general enquiries and requests for information.

# CONTENTS

## Executive Summary and Statement of Reasons

This Proposal has been prepared in order to consider the issues associated with the use of *Nicotiana* species in foods and, if necessary, to review the current food standards in relation to this matter in order to ensure that public health and safety is adequately protected. The current food standards are silent in relation to the use of *Nicotiana* species in food.

Many commonly and widely consumed vegetables of the nightshade family (Solanaceae) such as potatoes, tomatoes, eggplants and capsicums naturally contain low levels of nicotine. Nicotine has also been detected in cauliflower and tea – two non-solanaceous plants. Recently there have been a number of attempts overseas to deliver nicotine medications presented as food, such as in bottled water or in lollipops.

A concern expressed by health authorities is that the addition of tobacco or nicotine in food may promote or legitimise the smoking of tobacco or the use of smokeless tobacco products.

The objectives of this Proposal are to ensure that food regulations in relation to tobacco or any substance derived from tobacco in food are consistent with section 10 objectives of the FSANZ Act and with the principles of minimal effective regulation.

A report on the safety of nicotine has been prepared. Nicotine at the exposure levels obtained from tobacco smoke, is a powerful psychoactive drug. Nicotine is the major cause of the behavioural effects of tobacco and is responsible for some of its physiological effects. Human use of nicotine from tobacco meets the criteria for a drug of dependence.

The intestinal bioavailability of nicotine is low (approximately 20%) compared to the high absorption of nicotine from cigarette smoke (approximately 90%). Nicotine is rapidly and extensively distributed throughout the body, extensively metabolised, primarily in the liver, but also to a small extent in the lung, and excreted through the kidney.

The available data indicates that there are significant safety concerns associated with the use of nicotine in food, however, currently there are insufficient data to establish a safe level of intake for nicotine.

Three possible regulatory options for P278 were considered, namely:

1. Prohibit the use of *Nicotiana* species and all substances derived therefrom in all foods.

2. Allow the use of *Nicotiana* species, in all foods but restrict the level of nicotine to the level demonstrated to be safe.

3. Allow the use of *Nicotiana* species and all substances derived therefrom in all foods.

Option 1 provides the lowest cost regulatory approach while providing benefits to all parties identified in the impact analysis. Option 1 maintains consumer confidence in the safety and regulation of the food supply and maintains the delivery of a consistent government tobacco control message to the general community. Option 1 is the preferred regulatory option.

A total of fourteen submissions were received in response to the Draft Assessment Report (DAR), which was released for public comment in March 2004. Ten of the submissions were from Australia and four from New Zealand.

Twelve submissions strongly supported Regulatory Option 1 – Prohibit the use of *Nicotiana* species and all substances derived therefrom in all foods. Two submissions did not oppose the regulation of nicotine and *Nicotiana* species in food in principle, though one stated that consideration be given to the use of tobacco as a biofactory and the other stated that consideration should be given to the development of potentially reduced exposure products ('PREPs') intended to be offered as an alternative to smokers.

## Conclusion and Statement of Reasons

This Final Assessment Report agrees to the prohibition of the use of *Nicotiana* species and all substances derived therefrom in all food through the inclusion of *Nicotiana* species (tobacco) in Schedule 1 of Standard 1.4.4 – Prohibited and Restricted Plants and Fungi - for the following reasons:

- there are well recognised public health and safety risks associated with exposure to nicotine through smoking and the use of smokeless tobacco products;

- health authorities are concerned that the use of tobacco or nicotine in food may promote or legitimise the smoking of tobacco or the use of smokeless tobacco products;

- the cost to industry is likely to be minimal given that the use of tobacco and substances derived from tobacco in food is not widespread and the benefits of the proposed regulation outweigh the cost; and

- the proposed draft variation to the Code is consistent with the section 10 objectives of the FSANZ Act. In particular, it promotes public health and safety, and is based upon risk analysis using the best available scientific evidence.

# 1. Introduction

This Proposal has been prepared in order to consider the issues associated with the use of *Nicotiana* species in foods and, if necessary, to review the current food standards in relation to this matter in order to ensure that public health and safety is adequately protected.

In recent years, there has been an increase in both the number and extent of use of non-culinary herbs in orally consumed products presented as foods particularly beverages and energy bars. In some countries, this has included the use of tobacco plant extracts resulting in the development and marketing of nicotine containing bottled water and nicotine-containing lollipops and sweets.

# 2. Regulatory Problem

The current food standards are silent in relation to the use of *Nicotiana* species in food. Specifically, tobacco (*Nicotiana tabacum* L.) is neither expressly permitted nor expressly prohibited in food. Nicotine is not identified as a natural toxicant in Standard 1.4.1 – Contaminants and Natural Toxicants.

A concern expressed by health authorities is that the use of tobacco or nicotine in food may promote or legitimise the smoking of tobacco or the use of smokeless tobacco products.

This Proposal has been prepared by FSANZ under section 12AA of the FSANZ Act.

# 3. Objective

The objective of the Proposal is to determine whether food regulations are required in relation to the use of *Nicotiana* species. In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 10 of the FSANZ Act. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and

- any written policy guidelines formulated by the Ministerial Council.

# 4. Background

## 4.1 Historical Background

Smokeless tobacco products have been used worldwide for hundreds of years. In addition to tobacco, the products in some countries include a wide range of other constituents. The manner of use differs widely, although nearly all types of smokeless tobacco are used orally, with only a few rare types used nasally. More recently, this has included the use of tobacco plant extracts resulting in the development and marketing of nicotine-containing bottled water and nicotine containing sweets and lollipops.

Like smoked tobacco products, smokeless tobacco products are highly addictive due to the presence of nicotine. Nicotine levels from single doses of smokeless tobacco are similar to that from a cigarette, although the levels of nicotine rise faster and decrease slower in smokeless tobacco users. Nicotine is psychoactive, and users of smokeless tobacco show signs of addiction including a pattern of abuse involving escalating use, tolerance, and withdrawal symptoms.

During 2002, the Australian Government Department of Health and Ageing was alerted to the commercial importation of various smokeless tobacco products not covered by the *Customs (Prohibited Import) Regulations 1956*. Following a meeting of relevant agencies, it was agreed that FSANZ would investigate the need for specific regulations regarding tobacco and nicotine in food.

## 4.2 Current regulatory framework

### *4.2.1 Standard 1.4.4 – Prohibited and Restricted Plants and Fungi*

This Standard regulates some plants and fungi which may adversely affect human health. It lists the species of plants and fungi that must not be added to food or offered for sale as food. It also lists the species of plants and fungi that may not be used in food except as a source of a flavouring substance.

Schedule 1 in this Standard lists prohibited plants and fungi. This list, while not exhaustive, is based on known toxicity associated with these plants and fungi – these botanicals are considered to present a moderate to high public health and safety risk. There are many other plants and fungi which are not on this list which also present a high public health and safety risk, but these are not generally associated with food or inadvertent oral consumption.

Schedule 2 in this Standard lists those plants and fungi which are used as flavouring agents in food but which contain ingredients which are associated with some degree of toxicity. In these cases, a maximum level is applied to the toxic ingredient in the final food.
The maximum level of the ingredient is listed in the Table to clause 4 in Standard 1.4.1 – Contaminants and Natural Toxicants.

Standard 1.4.4 could be used to prohibit the use *Nicotiana* species in all or specified foods.

*4.2.2 Standard 1.5.1 – Novel Foods*

This is a broadly-based Standard, the purpose of which is to ensure that non-traditional foods that have features or characteristics that may raise safety concerns will undergo a risk-based safety assessment before they are offered for retail sale in Australia or New Zealand.

Novel Food is defined in the Standard as:

> *A non-traditional food or food ingredient for which there is insufficient knowledge in the broad community to enable safe use in the form or context in which it is presented, taking into account*
>
> *(a) the composition or structure of the product;*
> *(b) levels of undesirable substances in the product;*
> *(c) the potential for adverse effects in humans;*
> *(d) traditional preparation and cooking methods; or*
> *(e) patterns and levels of consumption of the product.*

Non-traditional food means a food which does not have a history of significant human consumption by the broad community in Australia or New Zealand.

This Standard could be used to regulate the use of food ingredients derived from those *Nicotiana* species that would be regarded as non-traditional foods. Safety considerations relating to the presence of nicotine could be addressed through this Standard by identifying nicotine as a contaminant and establishing a maximum level in the food in Standard 1.4.1 – Contaminants and Natural Toxicants.

*4.2.3 Standard 1.4.1 – Contaminants and Natural Toxicants*

This is a broadly-based Standard, that sets out the maximum level (ML) of specified metal and non-metal contaminants and natural toxicants in nominated foods. As a general principle, regardless of whether or not a ML exists, the level of contaminants and natural toxicants in all foods should be kept as low as reasonably achievable.

Maximum levels have been set at levels that are consistent with public health and safety and which are reasonably achievable from sound production and natural resource management practices. Control of nicotine in food could be achieved by including the substance in the Table to clause 5 – Maximum level of other natural toxicants in food.

## 4.3 Regulation in other countries

*4.3.1 USA*

In the USA, orally-consumed products are regulated as foods, dietary supplements or drugs. Herbs and foods containing herbs are generally regarded as dietary supplements and are regulated under the *Dietary Supplement Health and Education Act 1994* (DSHEA). According to this Act, *dietary supplements* are products *intended to supplement the diet to enhance health* and include *vitamins, minerals, amino acids, herbs and other botanicals*.

A dietary supplement is *not represented as a conventional food or a sole item of a meal or the diet*. Under this Act, herbal products can be sold without a safety or efficacy review by the FDA.

In the USA, there have been a number of attempts to market nicotine containing lollipops, lip balm and bottled water through various different distribution channels including the internet under the auspices of the DSHEA. In all cases, the FDA removed the products from the market because the products contain a drug that has not been approved by the FDA.

### *4.3.2 New Zealand*

In New Zealand, orally-consumed products are regulated as foods, dietary supplements or medicines. Dietary supplements are regulated under the Dietary Supplements Regulations 1985 (NZDSR). Under the NZDSR, a dietary supplement is defined as *any amino acids, edible substances, foodstuffs, herbs, minerals, synthetic nutrients and vitamins sold singly or in mixtures in controlled dosage forms as cachets, capsules, liquids, lozenges, pastilles, powders or tablets, which are intended to supplement the intake of those substances normally derived from the diet*.

Most of the products containing herbal substances (other than culinary herbs) would be regulated under the NZDSR. The NZDSR are likely to be reviewed in the near future and products regulated under these regulations to be regulated as either foods or medicines.

A prohibition for *Nicotiana* species under the Code would be consistent with the current restrictions on the sale of tobacco and tobacco products under the *Smokefree Environments Act 1990* and provisions for nicotine under the *Medicines Act 1981* and general prohibitions on harmful foods under the *Food Act 1981*. However, prohibitions under Standard 1.4.4 of the Code do not apply directly to products sold under the NZDSR, although the *Food Act 1981* harmful food provisions (see paragraph 9 (4)(b)) apply to all food for sale including dietary supplements.

### *4.3.3 Canada*

In Canada, orally-consumed products until recently were regulated as foods or drugs. Nicotine is included in Schedule F, which is a list of medicinal ingredients, the sale of which are controlled specifically by the Food and Drug Regulations. Specifically this regulation relates to *nicotine and its salts, for human use, except:*

- *in natural substances;*
- *in the form of chewing gum containing 4 mg or less of nicotine per dosage unit;*
- *in the form of a transdermal patch with a delivery rate of 22 mg or less of nicotine per day; or*
- *in a form to be administered orally by means of an inhalation device delivering 4 mg or less of nicotine per dosage unit.*

### *4.3.4 European Union*

There is no uniform legislation in the EU to regulate the use of herbs or food products containing herbs at this time. A preliminary draft proposal for a regulation of the European Parliament and of the European Council on the addition of vitamins and minerals and of certain other substances to food (SANCO/329/03) proposes to address the issue of the addition of nicotine to foods by placing the substance in Annex 3 - *Substances whose use in foods is prohibited or subject to conditions*; Part C – *Prohibited substances and ingredients containing them*.

## 4.4 Other relevant tobacco and nicotine regulations in Australia and New Zealand

Tobacco and nicotine in Australia and New Zealand is subject to considerable regulation. A review of relevant regulations indicates that tobacco and nicotine is not clearly regulated when presented as a food.

### *4.4.1 Smokefree Environments Act 1990 (NZ)*

The *Smokefree Environment Act 1990* defines tobacco products thus:

> *tobacco products means any product manufactured from tobacco and intended for use by smoking, inhalation, or mastication; and includes nasal and oral snuff; but does not include any medicine (being a medicine in respect of which there is in force a consent or provisional consent under section 20 or section 23 of the Medicines Act 1981) that is sold or supplied wholly or principally for use as an aid in giving up smoking*:

and section 29 reads

Tobacco product not to be advertised or labelled as suitable for chewing, etc.,

*(1) No person shall publish an advertisement for a tobacco product that directly or indirectly states or suggests that the product is suitable for chewing or for any other oral use (other than smoking).*

*(2) No person shall import for sale, sell, pack, or distribute any tobacco product labelled or otherwise describe as suitable for chewing, or for any other oral use (other than smoking).*

Consequently, the Act prevents the advertising, sale, distribution etc of tobacco products for any oral use other than smoking (unless it is an approved medicine under the *Medicines Act 1981* or used as an aid in giving up smoking). The Act does not cover products which contain nicotine but not tobacco such as nicotine water or nicotine lollipops.

### *4.4.2 Smokeless tobacco regulation in Australia*

A ban on the sale of oral snuff and chewing tobacco has been in place since 1989 under the *Trade Practices Act 1974*. The intention of the underlying policy was primarily to prevent mass importation and distribution of smokeless tobacco products.

The ban was pre-emptive, but feasible because of the relatively small number of consumers of these products in Australia. It is estimated that there are between 10,000 and 20,000 consumers of smokeless tobacco product in Australia.

The *Customs (Prohibited Imports) Regulations 1956* prohibit 'chewing tobacco, and snuffs intended for oral use, imported in an amount weighing more than 1.5 kg' (Schedule 12, Subregulation 4U (1)). However, importation for personal use is permitted:

- a permit is *not* required for the importation of chewing tobacco and oral snuff for quantities *less* than 1.5 kg;
- a permit is required for individual consumers with consignments of chewing tobacco and oral snuff greater than 1.5 kg. A maximum limit per permit has not been formally established, however, Department of Treasury officers do not issue permits for consignments in excess of 5 to 6 kg.

Though the Act regulates chewing tobacco, and snuffs intended for oral use, the Act does not cover products that contain nicotine but not tobacco, such as nicotine water and nicotine lollipops.

*4.4.3 Tobacco and nicotine as a therapeutic goods in Australia and New Zealand*

In Australia, the *Therapeutic Goods Act 1989* defined products that are regulated as foods or therapeutic goods.

therapeutic goods *means goods*:

*(a) that are represented in any way to be, or that are, whether because of the way in which the goods are presented or for any other reason, likely to be taken to be:*

*(i) for therapeutic use; or*
*(ii) for use as an ingredient or component in the manufacture of therapeutic goods; or*
*(iii) for use as a container or part of a container for goods of the kind referred to in subparagraph (i) or (ii); or*

*(b) included in a class of goods the sole or principal use of which is, or ordinarily is, a therapeutic use or a use of kind referred to in subparagraph (a) (ii) or (iii); and includes medical devices and goods declared to be therapeutic goods under an order in force under section 7, but does not include:*

*.....*

*(e) goods (other than goods declared to be therapeutic goods under an order in force under section 7) for which there is a prescribed standard in the Australia New Zealand Food Standards Code as defined in subsection 3(1) of the Food Standards Australia New Zealand Act 1991.....*

In New Zealand, orally-consumed products are regulated as foods, dietary supplements or medicines. A 'medicine' is a substance or article which is imported, sold, manufactured or supplied wholly or principally to treat a human being for a therapeutic purpose.

The term 'therapeutic purpose' covers a wide range of conditions and includes treating or preventing disease, altering the shape, structure, size or weight of the human body and preventing or interfering with the normal operation of a physiological function such as by increasing/decreasing its rate or through any other effect. It also includes cleaning, soaking or lubricating contact lenses, effecting contraception or inducing anaesthesia. The term also covers pregnancy test kits.

4.4.3.1 *Therapeutic Goods Administration Act 1989* (Australia)

The TGA Approved Terminology for Medicines, 1995, states that tobacco (*Nicotiana tabacum)* is 'registrable'. This requirement appears to be on the basis of the nicotine content of the plant, reported to be as high as 20,000-40,000 ppm in the leaf.

The Act covers the use of tobacco and nicotine in therapeutic goods and does not regulate the use of tobacco and nicotine presented in foods.

4.4.3.2 *Medicines Act 1981* (New Zealand)

The Medicines Control Agency have reclassified nicotine patches, lozenges and higher strength chewing gum as general sale list medicines. Nicotine replacement therapy is available as nicotine patches and nicotine gum (both over-the-counter at pharmacies), nicotine nasal spray (prescription medicine), nicotine inhaler (pharmacist only).

*4.4.4 Regulation of nicotine as a poison in Australia*

Nicotine is included in various schedules of the *Standard for Uniform Scheduling of Drugs and Poisons* (SUSDP) which is incorporated into State and Territory poisons legislation. Nicotine is a Schedule 6 substance when in preparations containing 3% or less nicotine when labelled and packed for the treatment of animals.

Nicotine is also in Schedule 7 of the SUSDP (dangerous poison) except when it is used as an aid for the withdrawal from smoking or when it is included in tobacco prepared and packed for smoking. There is no cut-off for substances included in Schedule 7, thus any preparation containing nicotine (including *Nicotiana tabacum*) is considered to be a dangerous poison (unless covered by other schedules or smoked).

Though nicotine is in Schedule 7 (dangerous poison) – under Appendix A, food is exempt from the SUSDP except *food additives before incorporation into food; or when used as a means of administering a poison for therapeutic use*.

# 5. Relevant Issues

## 5.1 Safety assessment of nicotine

A detailed report on the safety of nicotine is provided at Attachment 2.

Nicotine at the exposure levels obtained from tobacco smoke, is a powerful psychoactive drug. Nicotine is the major cause of the behavioural effects of tobacco and is responsible for some of its physiological effects. Human use of nicotine from tobacco meets the criteria for a drug of dependence.

The intestinal bioavailability of nicotine is low (approximately 20%) compared to the high absorption of nicotine from cigarette smoke (approximately 90%). Nicotine is rapidly and extensively distributed throughout the body, extensively metabolised, primarily in the liver, but also to a small extent in the lung, and excreted through the kidney.

The safety assessment demonstrates developmental, reproductive and cardiovascular effects of nicotine administration in animal studies. Genotoxicity studies on nicotine indicate weakly positive activity. There is evidence of cardiovascular effects in human studies. Although identified as a potential risk factor, currently there are a lack of data with respect to the effect of nicotine on human pregnancy. The available data indicates that there are significant safety concerns associated with the use of nicotine, however, currently there are insufficient data to establish a safe level of intake for nicotine.

## 5.2 Occurrence of nicotine in food

Many commonly and widely consumed vegetables of the nightshade family (Solanaceae) such as potatoes, tomatoes, eggplants and capsicums naturally contain low levels of nicotine. Nicotine has also been detected in cauliflower and tea – two non-solanaceous plants.

There are only five reports on the nicotine content of food plants. Four of these analytical studies, based either on GC-MS or a radioimmunoassay, have a low limit of detection and have found rather similar very low levels of nicotine in the five investigated food plants (Castro and Monji 1986 ; Davis et. al., 1991 ; Domino et. al., 1993 ; Siegmund et. al., 1999). A fifth study measured nicotine by gas-liquid chromatographic (GLC) (Sheen 1988) and reported significantly higher nicotine concentrations in foods. The differences may be due to methodological and contamination issues (Andersson et. al., 2003).

**Table 1. Nicotine content (μg/kg) in tomato, potato, eggplant, cauliflower and peppers**

| **Food source nicotine (μg/kg)** | **Castro and Monji, 1986** | **Davis et. al., 1991** | **Domino et. al., 1993** | **Siegmund et. al., 1999** |
|---|---|---|---|---|
| Potato | not included | Potato flesh: 15.3±1.7 (n=6) Potato peel: 4.8±0.8 (n=6) | 7.1±5.9 (n=11) | Raw potato: 4.5±2.2 (n=6) |
| Processed potato | not included | not included | not included | French fries: 11.5 and 6.9 |
| Tomato | 6.0±2.4 (n=6) | market tomato, 5.1±0.8 (n=3) fresh tomato, 9.6±2.7 (n=6) | 4.1±1.8 (n=8) | 2.4±1.2 (n=7) |
| Unripe tomato | 42.3; 14.2; 8.9; 25.3 | not included | not included | 16.1; 8.2; 6.8; 8.5; 6.8; 8.7; 7.0 |
| Processed tomato products | peeled tomato, 52; tomato paste, 11; tomato sauce, 3. | not included | not included | tomato paste, 5.3; tomato sauce, 4.5; tomato ketchup, 7.3 |
| Eggplant | 100 | n.d. (n=6) | not included | 1.9±0.7 (n=4) |
| Cauliflower | not included | 16.8±7.8 (n=6) | 3.8±2.2 (n=16) | not included |
| Green pepper | 5.7±0.0 (n=2) | n.d. (n=6) | not detected | 3.7; 5.8; 6.1 |
| Yellow Pepper | not included | not included | not included | 9.0 (n=1) |
| Red pepper | not included | not included | not included | 5.9 (n=1) |
| Green Pepperoni | not included | not included | not included | 8.7; 6.3 (n=2) |

The investigations summarised in Table 1 indicate that certain edible food plants contain relatively low amounts of nicotine, generally below 10 μg/kg for fresh fruits. The ripeness of foods has been demonstrated to influence nicotine content; the highest content of nicotine found in a ripe tomato was 9.8 μg/kg fresh weight, whereas the highest content of nicotine in the green tomato was 42.3 μg/kg fresh weight (Castro and Monji 1986). Nicotine appears to survive a variety of processing operations such as the preparation of tomato ketchup, sauces, and pastes as well as frying and boiling of potatoes. Processed products showed slightly higher nicotine concentrations in comparison to the related fresh fruit, possibly a result of the higher percentage of dry matter (Siegmund et. al., 1999).

Relatively high concentrations of nicotine were found in tea leaves (Table 2) though this was not reflected in brewed tea (Siegmund et. al., 1999). Nicotine content was higher in regular than in decaffeinated tea and higher in instant tea than tea brewed from tea leaves (Davis et. al., 1991). There were large variations in nicotine concentration found within types of black tea, whereas concentrations of nicotine were more or less consistent within green teas. The extraction yield of nicotine in brewed tea is in the range of 20-25% (Siegmund et. al., 1999).

**Table 2: Nicotine concentrations (μg/kg) in tea leaves (Siegmund et. al., 1999)**

| Sample | nicotine (μg/kg) ww | nicotine (μg/kg) dw |
|---|---|---|
| Earl Grey – black tea | 381 | 404 |
| Ceylon Orange Pekoe – black tea | 164 | 174 |
| Assam –black tea | 1593 | 1696 |
| Darjeeling – black tea | 812 | 864 |
| China Fancy Gunpowder – green tea | 317 | 337 |
| Earl Grey – green tea | 358 | 380 |
| Formosa Gunpowder – green tea | 470 | 499 |
| Temple of Heaven – green tea | 337 | 353 |

Nicotine biosynthesis in tea plants (*Camellia sinensis*) has not been demonstrated; however its presence in tea products has been consistently found (Davis et. al., 1991 ; Sheen 1988 ; Siegmund et. al., 1999). A possible explanation for the presence of nicotine in tea samples is the contamination of the tea through the use of nicotine as a pesticide (Andersson et. al., 2003).

### 5.3 Addition of nicotine medications to food

Recently there have been a number of attempts to deliver nicotine medications in foods such as in water and lollipops. Two nicotine-containing products presented as foods, though sold dietary supplements, have been identified and were sold in the USA through a variety of different distribution channels including the Internet:

- Nicotine water marketed by S&F Garret and QTF Inc. Bottled water with added nicotine sold as a dietary supplement (2 mg or 4 mg per bottle – 1 to 2 cigarette equivalence). Sold over the Internet since 2000, sale in US stores of 'Nico Water' were planned for July 2002 by QTF Inc.

- Nicotine lollipops marketed by Bird's-Hill Pharmacy, Ashland Drug and Compounding Pharmacy. Presented as sugar-free lollipops in assorted flavours containing nicotine salicylate in dosages from 0.5-4 mg. Lollipops sold under brand names such as NicoStop, NicoPop and Likatine. Sold in three independent US pharmacies and over the Internet since 2001.

The US FDA ordered a stop to sales of these products in 2002 stating that nicotine containing products sold as 'dietary supplements' were unapproved new drugs, dispensed without a prescription, without adequate directions and without warning labels.

In Australia and New Zealand use of nicotine at physiological and/or therapeutic levels to aid in the cessation of smoking is covered under the appropriate therapeutics and medicines regulations.

### 5.4 Nutritional aspects of tobacco proteins

Tobacco has been promoted as a potentially important food crop in combination with its traditional uses of smoking and chewing. As a food crop, tobacco grown in dense spacing could produce about four times more protein per acre than soybeans. Protein can be readily extracted from tobacco resulting in the isolation of two protein fractions: Fraction 1 (rubisco) and Fraction 2 proteins (Wildman, 1983; Kwanyuen et. al., 2002).

Crystalline Fraction 1 and 2 proteins are water soluble; tasteless and odourless; and can be isolated to a high degree of purity. There is also evidence demonstrating that the nicotine concentration of crystallised Fraction 1 protein is below the amount found naturally in tomatoes, eggplants, capsicums and tea (i.e. <3-10 μg/kg) (Wildman, 1983 ; Siegmund et. al., 1999), although such information is lacking for Fraction 2 proteins. The functional properties of these proteins, such as 'heat set', are similar to egg albumin and casein.

The nutritional quality of tobacco proteins can be determined by assessing their protein digestibility-corrected amino acid score (PDCAAS). The PDCAAS is a useful measure of protein quality, as it can be determined from basic food/protein composition data in conjunction with digestibility information. The main limitation in using this score is its inability to reflect how the human body uses protein following its digestion; e.g., the body can sometimes adapt to very low levels of certain essential amino acids during long term exposure to a diet consisting of poor quality proteins (FAO, 1991). Despite this limitation, the PDCAAS has good compatibility with other more thorough biological assays of protein quality (Wardlaw and Insel, 1993).

A PDCAAS has been calculated for Fraction 1 and 2 proteins by FSANZ in accordance with the method established by the United Nations Food and Agriculture Organization (FAO, 1991). Table 3 shows how the PDCAAS was calculated for Fraction 1 and 2 proteins, and was undertaken in accordance with the following procedure:

1. An amino acid score has been calculated for each essential amino acid by dividing the quantity (mg) of the amino acid in 1g of the Fraction 1 protein by the FAO amino acid requirement in mg/g crude protein for preschool children (2-5 years).

2. The lowest amino acid score represents the first limiting amino acid for the protein, which for Fraction 1 protein is a value of 1.0 based on lysine content, and Fraction 2 protein is a value of 0.0 based on tryptophan content.

3. The uncorrected amino acid score is multiplied by its digestibility expressed as a percentage (i.e. the percentage of ingested protein that is digested and absorbed by the intestine). A value of 100% (complete digestion) has been identified for Fraction 1 protein (Nguyen and Harvey, 2001), resulting in a final value that is equivalent to the uncorrected amino acid score – 1.0. A similar factor has not been investigated for Fraction 2 proteins.

4. The final value is multiplied by 100 to obtain the PDCAAS as a percentage: 1.0x100 = 100% for Fraction 1 proteins and 0% for Fraction 2 proteins.

**Table 3: Calculation of the protein-corrected amino acid scores for Tobacco proteins**

| | Amino Acid Requirement (mg/day)* | Fraction 1 Protein | | Fraction 2 Protein | |
|---|---|---|---|---|---|
| | | Amino Acid Profile (mg/1g protein)** | Amino Acid Score | Amino Acid Profile (mg/1g protein)** | Amino Acid Score |
| *Essential Amino Acid* | | | | | |
| Histidine | 19 | 22 | 1.2 | 50 | 2.6 |
| Isoleucine | 28 | 43 | 1.5 | 38 | 1.4 |
| Leucine | 66 | 88 | 1.3 | 41 | 0.6 |
| Lysine | 58 | 58 | 1.0 | 39 | 0.7 |
| Methionine + Cysteine | 25 | 46 | 1.8 | 49 | 2.0 |
| Phenylalanine + Tyrosine | 63 | 93 | 1.5 | 172 | 2.7 |
| Threonine | 34 | 52 | 1.5 | 46 | 1.4 |
| Tryptophan | 11 | 15 | 1.4 | 0 | 0.0 |
| Valine | 35 | 72 | 2.1 | 105 | 3.0 |
| *Calculations* | | | | | |
| Uncorrected Amino Acid Score | | | 1.0 | | 0.0 |
| Digestibility Factor | | | 100% | | |
| PDCAAS | | | 100% | | 0% |

* Amino Acid requirements from FAO, 1991.

** Amino acid profile from Wildman, 1983.

The PDCAAS shows that when consumed in an amount that meet the body's total protein needs, Fraction 1 protein is capable of supplying essential amino acids at 100% the human body's requirements for each individual amino acid. This assessment of quality is a good result for a protein isolate, and is a comparable to other protein isolates such as casein and soybean protein (PDCAAS = 100% and 92% respectively) (FAO, 1989). Fraction 2 protein however, is nutritionally poor.

Therefore, tobacco may yield a high volume of good quality protein (Fraction 1) that could have potential uses in the manufacture of food. This protein could represent a viable alternative to other established protein sources such as casein and soybean proteins.

## 5.5 Nutritional aspects of tobacco seed oil

Tobacco seed is a by-product of tobacco leaf production, and can be used to produce an edible oil, currently available for this purpose in some European countries. Tobacco seeds contain a high quantity of oil ranging from 33 to 40% of the total seed weight depending on the plant strain (Eshetu, 2000).

The nutritional value of tobacco seed oil can be best defined by assessing its fatty acid composition. The basic fatty acid profile in the seed oil has been identified as follows (expressed in % of total fatty acids): polyunsaturated = 76.1%, monounsaturated = 10.5%, saturated = 13.3%. A more detailed profile can be found in Table 4 below.

**Table 4: Fatty acid content of tobacco seed oil (Eshetu, 2000)**

| Fatty Acid | Quantity in Kentucky 104 strain (% of total fatty acids) | Quantity in Bright Italia strain (% of total fatty acids) | Quantity in Bright V strain (% of total fatty acids) | Mean quantity (% of total fatty acids) |
|---|---|---|---|---|
| Saturated | | | | |
| 16:0 | 9.5 | 9.2 | 8.9 | 9.2 |
| 17:0 | 0.1 | 0.1 | 0.1 | 0.1 |
| 18:0 | 2.8 | 2.5 | 2.6 | 2.6 |
| 20:0 | 1.1 | 1.4 | 1.1 | 1.2 |
| 22:0 | 0.2 | 0.2 | 0.2 | 0.2 |
| Saturated fat Subtotal | 13.7 | 13.4 | 12.9 | 13.3 |
| Monounsaturated | | | | |
| 18:1 | 10.6 | 9.5 | 11.1 | 10.4 |
| 16:1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Monounsaturated fat subtotal | 10.7 | 9.6 | 11.2 | 10.5 |
| Polyunsaturated | | | | |
| 18:2 | 0.6 | 0.8 | 0.7 | 0.7 |
| 18:3 | 74.9 | 76.1 | 75.1 | 75.4 |
| Polyunsaturated fat subtotal | 75.5 | 76.9 | 75.8 | 76.1 |
| Subtotals combined | 99.9 | 99.9 | 99.9 | 99.9 |

The fatty acid composition of tobacco seed oil is comparable to sunflower, safflower and corn oils (Wardlaw and Insel, 1993).

From a public health perspective, the low saturated fatty acid proportion is desirable, as a high intake of saturated fat has been identified as a significant risk factor in the development of cardiovascular disease. Therefore, tobacco seed oil could be considered nutritionally appropriate for use in the manufacture of foodstuffs, particularly in comparison to high saturated fat vegetable oils that are commonly added to foods, such as palm oil and coconut oil (51% and 87% of total fatty acids are saturated respectively) (Mann and Truswell, 2002; Wahlqvist, 1997).

## 5.6 Regulation of nicotine as a pesticide

Tobacco has been used as a pesticide for over 300 years. Tobacco and nicotine are still used internationally as pesticides. There are currently six nicotine-containing pesticides registered with the USA EPA. Nicotine-containing pesticides are primarily used in domestic gardening and organic farming applications.

### *5.6.1 Regulation of nicotine as a pesticide in Australia*

Nicotine and its salts are not registered with the APVMA. In Australia, the National Standard for Organic and Bio-dynamic Produce excludes the use of tobacco extracts from the permitted material for plant and animal pest and disease control.

The Code does not contain maximum residue limits for nicotine and nicotine sulphate.

### *5.6.2 Regulation of nicotine as a pesticide in New Zealand*

There has not been any New Zealand-registered pesticide (organic or otherwise) containing nicotine since 1975. Nicotine is not permitted under the NZFSA organic standard.

Nicotine is not listed in the *New Zealand (Maximum Residue Limits of Agricultural Compounds) Food Standards 2004*. However, in New Zealand, Subsection 8 (b) of the standard states:

*A person may sell a food containing residues of an agricultural compound not exceeding 0.1 mg/kg where:*

*(i) That agricultural compound is not specified in column one of the First Table of these standards; or*

*(ii) The food is not specified or is not of a type, kind or class specified in column two of the First Table in the row corresponding to the agricultural compound specified in column one of the First Table.*

Thus the New Zealand MRL standard does not prohibit nicotine residues no greater than 0.1 mg/kg.

## 5.7 *Nicotiana* species as a source of food ingredients

The introduction of genes into plants like tobacco, corn, soybeans and alfalfa to enable them to produce and accumulate new substances has been possible for many years. An abundance of scientific literature documents the successful production in tobacco of protein pharmaceuticals, vaccines and other medicinals, enzymes, polymers and food ingredients. A variety of technologies have been developed to make these 'plant factory' systems possible, and the leading commercial research is now focused on optimizing the production systems and post-harvest bio-processing aspects to the required standards.

Three studies have shown that it is possible to influence the nicotine alkaloid levels of Solanaceous plants by genetic modification and this possibility should be considered in the risk assessment of modified Solanaceous plants varieties (Andersson et. al., 2003).

- Addition of a bacterial lysine decarboxylase gene to root cultures of *N. tabacum* demonstrated up to a 3-fold increased level of the nicotine alkaloid anabasine in some overexpressed cell lines (Feckler et. al., 1993).

- Over-expressing a yeast ornithine decarboxylase gene in transgenic roots of *N. rustica* can lead to a 2-fold increase nicotine accumulation (Hamill et. al., 1990).

- Addition of the gene for *Vitreoscilla* haemoglobin (VHb) introduced and expressed in *N. tabacum* contained on average, 30-40% more chlorophyll and 34% more nicotine than controls (Holmberg et. al., 1997).

# 6. Regulatory Options

Possible regulatory options for P278 are given below.

1. Prohibit the use of *Nicotiana* species and all substances derived therefrom in all foods.

2. Allow the use of *Nicotiana* species, in all foods but restrict the level of nicotine to the level demonstrated to be safe.

3. Allow the use of *Nicotiana* species and all substances derived therefrom in all foods.

## 6.1 Regulatory issues raised in response to the Initial Assessment Report

The Initial Assessment Report proposed the three regulatory options identified above. All submitters recognised the role of FSANZ in regulating food products. However two submitters, British American Tobacco Australia Ltd (BATA) and VicHealth Centre Tobacco Control (VicHealth) stated that the Proposal highlighted the major problems with the current tobacco regulatory environment.

VicHealth proposed a modified Option 2 – Allow the use of *Nicotiana* species in all foods but restrict the level of nicotine to the level demonstrated to be safe and not to be therapeutic or psychoactive. The option was raised to prevent foods such as the nightshades, known to naturally contain low levels of nicotine, from being banned. Option 1 in this Proposal addresses the concerns of VicHealth by preventing the addition of nicotine to food, while permitting the sale of foods that naturally contain nicotine, such as the nightshades.

BATA supports sensible regulatory measures by public health authorities to reduce public health impact of tobacco, which they believe is consistent in principle with FSANZ's proposal to regulate nicotine in food. However in pursuing this objective BATA strongly recommend that FSANZ does not assume oversight for an area more appropriately the purview of tobacco control authorities.

## 6.2 Regulatory issues raised in response to the Draft Assessment Report

A total of fourteen submissions were received in response to the Draft Assessment Report (DAR), which was released for public comment in March 2004. Ten of the submissions were from Australia and four were from New Zealand. Twelve submissions strongly supported Regulatory Option 1 – Prohibit the use of *Nicotiana* species and all substances derived therefrom in all foods. Two submissions did not oppose the regulation of nicotine and *Nicotiana* species in food in principle, though one stated that consideration be given to the use of tobacco as a biofactory and other stated that consideration should be given to the development of potentially reduced exposure products ('PREPs') intended to be offered as an alternative to smokers. Issues raised by four submitters are addressed below.

### *6.2.1 Nicotine from sources other than Nicotiana species*

New Zealand Food Safety Authority (NZFSA) while supporting Option 1 noted that there is no clear statement in the Draft Assessment Report about the likelihood of nicotine being produced from sources other than *Nicotiana* species, for example synthetic sources or nicotine from other plant species. Such nicotine would not be prohibited by option 1 alone.

#### 6.2.1.1 Consideration by FSANZ

Although methods for the chemical synthesis of nicotine are available, nicotine is primarily extracted from the dried leaves of *Nicotiana tabacaum* and *Nicotiana rustica* where it occurs to the extent of 2 to 8%, combined with citric and malic acids. A review of information available to FSANZ has been unable to find evidence of nicotine extracted from plant sources other than *Nicotiana* species or derived from synthetic sources being traded commercially. In the absence of such information FSANZ believes the issue is best addressed by proceeding with the preferred option, option 1.

### *6.2.2 Occurrence of nicotine in food products*

Cadbury Schweppes Pty Ltd while supporting Option 1, acknowledges that a number of food products where nicotine is present in natural forms and recommends that those foods are included in Standard 1.4.1, Clause 3 'Maximum levels of non-metal contaminants in food' along with a corresponding maximum level of permitted nicotine.

#### 6.2.2.1 Consideration by FSANZ

The occurrence of nicotine in food products was reviewed in section 5.2 of this Report. Five reports on the nicotine content of food plants were reviewed. The data presented in these reports were informative in identifying the presence of nicotine in certain food plants. The data indicated that certain edible food plants contain relatively low amounts of nicotine and that nicotine appears to survive a variety of processing operations. The data however, is insufficient to support recommendations corresponding to maximum levels of permitted nicotine associated with nominated food products. This regulation will prohibit the addition of nicotine from tobacco to food and not regulate endogenous sources of nicotine present in food plant.

### *6.2.3 Nicotiana species as a source of food ingredients*

Department of Primary Industries and Fisheries (Queensland) appreciates that the use of tobacco and nicotine in food may promote or legitimise the smoking of tobacco or the use of smokeless tobacco products however highlights that tobacco is one of the prime candidates for use as a biofactory and therefore suggests that recommendations should allow legitimate opportunities to be considered and not be ruled out by a blanket recommendation.

#### 6.2.3.1 Consideration by FSANZ

The proposed regulation will not rule out the opportunity to use *Nicotiana* species as a biofactory or as a source of food ingredients. If a benefit is established for consuming a food derived from *Nicotiana* species, this can be assessed for inclusion in the Code as a novel food.

The issues of safe nicotine levels and any other toxic entities in such foods would then need to be addressed.

#### *6.2.4 Potentially reduced exposure products (PREPs)*

British American Tobacco Australia (Limited) (BATA), which in principle does not oppose the regulation of the use of nicotine and *Nicotiana* species in food, believes that any proposed regulation in this regard, must give due consideration to, and make clear provision for developments of potentially reduced exposure products ('PREPs') intended to be offered as an alternative to smokers choosing to potentially minimise the risks associated with smoking.

##### 6.2.4.1 Consideration by FSANZ

The proposed regulation will not prevent developments of potentially reduced exposure products (PREPs) which may be used orally, intended to be offered as an alternative to smokers choosing to potentially minimise the risks associated with smoking. Discussions with BATA indicated that PREPs, used for the purposes of reducing the risks of associated with smoking, would not be considered foods and are likely to be regulated by the TGA.

# 7. Impact Analysis

## 7.1 Affected Parties

Parties that are potentially affected by this Proposal include:

- Those sectors of the food industry wishing to use *Nicotiana* species and all substances derived therefrom, including nicotine, in all foods.
- Traditional consumers of food products derived from *Nicotiana* species including nicotine free tobacco seed oil.
- Australian and New Zealand Governments involved in nicotine and tobacco control.
- Public health workers involved in nicotine and tobacco control.

## 7.2 Data Collection

The safety assessment of nicotine (Attachment 2), the public health and concerns regarding the promotion of nicotine consumption in food, the regulation of nicotine containing pesticides in Australia and New Zealand and public submissions have been used to inform the impact analysis of various regulatory and non-regulatory options.

## 7.3 Impact Analysis

#### *7.3.1 Option 1 - Prohibit the use of Nicotiana species and all substances derived therefrom in all foods*

#### 7.3.1.1 Industry

All submissions from the food industry support this option. There will be little impact on the food industry as the practice of using tobacco and ingredients derived therefrom in food is not widespread. Examples of the type of products from overseas that would be affected by this regulation will include tobacco seed oil, 'tobacco whisky' and the cocktail drink known as the 'nicotini' made by soaking tobacco leaf in vodka. The regulation will not prevent products derived from the tobacco plant being assessed for inclusion as a novel food in the future. The regulation will maintain consumer confidence in the safety and regulation of the food supply.

#### 7.3.1.2 Consumers

Prior to the initiation of the Proposal, FSANZ fielded several media enquiries regarding the use of nicotine in foods overseas. Though there have been a limited number of submissions from groups other than industry or government, the overwhelming sentiment associated with these submissions have been to support the prohibition of nicotine and tobacco in food. The regulation will prevent consumers from purchasing products such as tobacco seed oil, 'tobacco whisky' and the 'nicotini'. This regulation will be a minor inconvenience to a small number of people, as these products fill niche markets and are not widely consumed in Australia and New Zealand.

#### 7.3.1.3 Government

All submissions from government agencies support this option. There will be limited impact on government enforcement agencies. The regulation will further control access of tobacco products to the public. It will prevent the sale of oral tobacco products such as 'tobacco whisky' and the 'nicotini' and nicotine medications presented as foods. The regulation will maintain consumer confidence in the safety and regulation of the food supply and deliver a consistent government tobacco control message to the general community. The regulation would be the lowest cost solution.

#### 7.3.1.4 Health Professionals

There is currently little impact on health professionals associated with dietary exposure to tobacco and nicotine in fruits and vegetables given the low levels of intake in the Australian and New Zealand communities. The adverse health impact associated with the consumption products such as 'tobacco whisky' and the 'nicotini' will remain low, as the sale of these products, currently restricted to a small number of individuals, will be prohibited.

### *7.3.2 Option 2 - Allow the use of Nicotiana species, in all foods but restrict the level of nicotine to the level demonstrated to be safe*

#### 7.3.2.1 Industry

The available data, outlined in Attachment 2, indicates that there are significant safety concerns associated with the use of nicotine. There is currently insufficient data to establish a safe level for the intake for nicotine.

There would be a large cost to industry to pay for the provision of additional toxicological information supporting the level of nicotine in food demonstrated to be safe. There would be a significant cost to industry to demonstrate to the general public that the product would be safe.

7.3.2.2 Consumers

Though there have been a limited number of submissions from groups other than industry or government, the overwhelming sentiment associated with these submissions have been to support the prohibition of nicotine and tobacco in food. Specifically one submission to the IAR from the Women's Christian Temperance Union objects to nicotine being added to any food products. Addition of tobacco or nicotine to foods, in any quantities, may result in considerable consumer distrust in food products associated with tobacco and/or nicotine.

7.3.2.3 Government

The available data indicates that there are significant safety concerns associated with the use of nicotine, however, there is currently insufficient data to establish a safe level of intake for nicotine. All submissions from government agencies oppose this option. This option would significantly impact on government agencies. There may be difficult scientific, technical and social issues associated with the identification of a safe level of nicotine in food. The regulation will present an inconsistent government tobacco control message to the general community. A concern expressed by health authorities is that the use of tobacco or nicotine in food may promote or legitimise the smoking of tobacco. There may be revenue issues associated with this regulation, as food products containing tobacco may not be recognised by the current smokeless tobacco regulations.

7.3.2.4 Health Professionals

If it is possible to set a safe level for nicotine in food, there will be little direct effect on health professionals. Indirect effects may include increased use of tobacco products associated with the delivery of an inconsistent government tobacco control message to the general community. Increased tobacco use will significantly impact on health resources.

*7.3.3 Option 3 - Allow the use of Nicotiana species and all substances derived therefrom in all foods.*

7.3.3.1 Industry

This regulation would impose significant costs to the Australian and New Zealand food industry. The general community would lose of confidence in the safety of the food supply. The cost to industry may be in the form of loss of market share and loss of export opportunities.

7.3.3.2 Consumers

There have been a limited number of submissions from groups other than industry or government. The overwhelming sentiment associated with these submissions have been to support the prohibition of nicotine and tobacco in food.

7.3.3.3 Government

This report has demonstrated that there may be significant public health and safety issues associated with general permission for nicotine use in food. All submissions from government agencies oppose this option. There would be a significant impact on government agencies. This regulation would be contrary to section 10 of the FSANZ Act, specifically the protection of public health and safety. This option would foster a loss of community confidence in the food regulatory system. The regulation would be contrary to government initiatives that control access to tobacco in the community. There would be significant long-term health costs to the community.

7.3.3.4 Health Professionals

This regulation would propose a significant health and safety risk to the community. There would be significant long-term health costs to the community.

*7.3.4 Summary*

Option 1 - Prohibit the use of *Nicotiana* species and all substances derived therefrom in all foods – provides is the lowest cost regulatory approach while providing benefits to all parties identified in the impact analysis. Option 1 maintains consumer confidence in the safety and regulation of the food supply. Option 1 maintains the delivery of a consistent government tobacco control message to the general community. Option 1 is the preferred regulatory option.

# 8. Consultation

## 8.1 Submissions in response to the Initial Assessment Report

A total of eleven submissions were received in response to the Initial Assessment Report (IAR), which was released for public comment in October 2003. These submissions are summarised in Attachment 3. Eight of the submissions were from Australia and three were from New Zealand. Nine submissions strongly supported Regulatory Option 1 – Prohibit the use of *Nicotiana* species and all substances derived therefrom in all foods. One submission proposed a modified Regulatory Option 2 - Allow the use of *Nicotiana* species in all foods but restrict the level of nicotine to the level demonstrated to be safe and to not be therapeutic or psychoactive. One submission strongly recommends that FSANZ does not assume oversight for an area more appropriately the purview of tobacco control authorities.

## 8.2 Submissions in response to the Draft Assessment Report

A total of fourteen submissions were received in response to the Draft Assessment Report (DAR), which was released for public comment in March 2004. Ten of the submissions were from Australia and four were from New Zealand.
Twelve submissions strongly supported Regulatory Option 1 – Prohibit the use of *Nicotiana* species and all substances derived therefrom in all foods. Two submissions, in principle did not oppose the regulation of nicotine and *Nicotiana* species in food though believe that consideration be given to the use of tobacco as a biofactory and the development of potentially reduced exposure products ('PREPs') intended to be offered as an alternative to smokers. Issues raised by four submitters have been addressed in section 6.2 of this Report.

### 8.3 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

There are no relevant international standards and amending the Code to allow the control of *Nicotiana* species in foods is unlikely to have a significant effect on international trade as the practice of using tobacco or ingredients derived from tobacco in food is not widespread.

The proposed amendments to the Code are considered to be minor in nature and without significant trade implications. The matter therefore was not notified to the WTO under either the Technical Barrier to Trade or Sanitary and Phytosanitary Measure Agreements.

## 9. Conclusion and Recommendation

This Final Assessment Report agrees to the prohibition of the use of *Nicotiana* species and all substances derived therefrom in all food through the inclusion of *Nicotiana* species (tobacco) in Schedule 1 Standard 1.4.4 – Prohibited and Restricted Plants and Fungi – for the following reasons:

- there are well recognised public health and safety risks associated with exposure to nicotine through smoking and the use of smokeless tobacco products;
- health authorities are concerned that the use of tobacco or nicotine in food may promote or legitimise the smoking of tobacco or the use of smokeless tobacco products;
- the cost to industry is likely to be minimal given that the use of tobacco and substances derived from tobacco in food is not widespread and the benefits of the proposed regulation outweigh the cost; and
- the proposed draft variation to the Code is consistent with the section 10 objectives of the FSANZ Act. In particular, it promotes public health and safety, and is based upon risk analysis using the best available scientific evidence.

## 10. Implementation and review

A notification will now be made to the Ministerial Council and subject to any requests from the Ministerial Council for a review, the amendments to the Code with respect to Standard 1.4.4 – Prohibited and Restricted Plants and Fungi, would come into effect shortly thereafter upon gazettal.

## 11. References

Andersson, C., Wennström, P., Gry, J. 2003. Nicotine alkaloids in Solanaceous food plants. *TemaNord* 531: 1-37.

Castro, A., Monji, N. 1986. Dietary nicotine and its significance in studies on tobacco smoking. *Biomed. Arch.,* 2 (2): 91-97.

Davis, R.A., Stiles, M.F., de Bethizy, J.D., Reynolds, J.H. 1991. Dietary intake: a source of urinary cotinine. *Food Chem. Toxicol.,* 29 (12): 821-827.

Domino, E.F., Hornbach, E., Demana, T. 1993. Relevance of nicotine content of common vegetables to the identification of passive tobacco smoke. *Med. Sci. Res.,* 21: 571-572.

Eshetu B (2000); '*Nicotiana tabacum* L. Seed Oil'; http://ipp.boku.ac.at/pz/oilseeds/eshetu.html

Food and Agriculture Organization (FAO). 1991. Protein quality evaluation: Report of Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper Series No 5.1, FAO, Rome

Fecker, L.F., Rügenhagen, C., Berlin, J. 1993. Increased production of cadaverine and anabasine in hairy root cultures of *Nicotiana tabacum* expressing bacterial lysine decarboxylase gene. *Plant Mol. Biol.,* 23: 11-21.

Hamill, J.D., Robins, R.J., Parr, A.J., Evans, D.M., Furze, J.M., Rhodes, M.J.C. 1990. Over-expressing a yeast ornithine decarboxylase gene in transgenic roots of *Nicotiana rustica* can lead to enhanced nicotine accumulation. *Plant Mol. Biol.,* 15: 27-38.

Holmberg, N., Lilius, G., Bailey, J.E., Bulow, L. 1997. Transgenic tobacco expressing *Vitreoscilla* haemoglobin exhibits enhanced growth and altered metabolic production. *Nature Biotechnol.,* 15: 244-247.

Kwanyuen, P., Allina, S.M., Weissinger, A.K., Wilson, R.F. 2002. A new form of crystalline rubisco and the conversion to its common dodecahedral form. *J Proteome Res* 1(5): 471-473.

Mann, J., Truswell, S. 2002. Essentials of Human Nutrition, 2nd ed. Oxford University Press, New York. p401-402.

Nguyen, R.T., Harvey, H.R. 2001. Preservation of protein in marine systems: Hydrophobic and other noncovalent associations as major stabilizing forces. *Geochimica et Cosmochimica Acta* 65(9): 1467-1480.

SANCO/329/03. 17.01.2003. Preliminary Draft Proposal for a Regulation of the European Parliament and of the Council on the addition of vitamins and minerals and of certain other substances to food. Commission of the European Communities, Brussels, Belgium.

Siegmund, B., Leitner, E., Pfannhauser, W. 1999. Determination of the nicotine content of various edible nightshades (Solanaceae) and their products and estimation of the associated dietary nicotine intake. *J. Agric. Food Chem*, 47 (8): 3113-3120.

Sheen, S.J. 1988. Detection of nicotine in foods and plant material. *J. Food Sci.,* 53 (5): 1572-1573.

Walquist, M. 1997. Food and Nutrition. Allen & Unwin, Sydney. p210-212.

Warlaw, G.M., Insel, P.M. 1993. Perspectives in Nutrition. Mosby, Philadephia; p107-142.

Wildman, S. 1983. An Alternative Use For Tobacco Agriculture: Proteins For Food Plus A Safer Smoking Material in *Plants: The Potential for Extracting Protein, Medicines, and other Useful Chemical – Workshop Proceedings.* p 63-77; Washington D.C. www.wws.princeton.edu/cgi-bin/byteserv.prl/ ~ota/disk3/1983/8315/831504.PDF

## ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Safety assessment report on nicotine
3. Summary of submissions

**ATTACHMENT 1**

## Draft Variation to the *Australia New Zealand Food Standards Code*

**To commence: on gazettal**

**[1]** ***Standard 1.4.4*** *of the Australia New Zealand Food Standards Code is varied by inserting in* Schedule 1 –

| | |
|---|---|
| *Nicotiana* spp. | Tobacco |

## ATTACHMENT 2

# Safety Assessment of Nicotine


**SUMMARY**

*Introduction*
This report assesses the safety of nicotine. Nicotine in tobacco smoking concentrations, is a powerful psychoactive drug. Nicotine is the major cause of the predominant behavioural effects of tobacco and some of its physiological consequences. Human use of nicotine from tobacco meets the criteria for a drug of dependence.

Nicotine is a tertiary amide consisting of a pyridine and a pyrrolidine ring. Every part of the tobacco plant, except the seed, contains nicotine.

*Absorption, Distribution, Metabolism and Excretion (ADME)*

Nicotine is a water and lipid soluble drug, which in the free base form, is readily absorbed via respiratory tissues, skin and the gastrointestinal tract. The intestinal bioavailability of nicotine and associated 'first-pass' metabolism suggests the bioavailability of orally administered nicotine is low (approximately 20%). This contrasts with the high absorption of nicotine from cigarette smoke (approximately 90%).

Nicotine is rapidly and extensively distributed throughout the body. Thirty to 60 minutes after intravenous administration of nicotine to rats, nicotine concentrations two to 15 times higher than those in plasma have been observed in a number of organs, i.e. adrenals, liver, brain, lung, heart, gastro-intestinal tissue, spleen, thymus and kidney in addition to skeletal muscle. Nicotine freely crosses the placenta and has been found in amniotic fluid, umbilical cord blood and the fetus. Nicotine has been found to concentrate in breast milk, with milk:serum concentration ratios averaging 2.9 in a group of nursing mothers; the nursed infants serum:maternal serum nicotine concentration ratio averaging 0.06. High concentrations of nicotine have been observed in the salivary gland, the concentration ratio of nicotine in saliva:plasma generally exceeds 10.

Nicotine is rapidly and extensively metabolised, primarily in the liver, but also to a small extent, in the lung. Nicotine is excreted partially unchanged by the kidney, but largely in the form of 20 or more different metabolites, which contain an intact pyridine ring. The primary metabolite of nicotine in most species is cotinine. Cotinine has a longer elimination half-life than nicotine (15 h compared with 2 h) although there is considerable individual variability.

*Safety assessment of nicotine*
The assessment of safety of nicotine summarises several, reproductive, developmental, and cardiovascular studies conducted in animals. Genotoxicity studies on nicotine were reviewed. Human studies presented focussed on the cardiovascular effects and toxicity of nicotine. The limited data associated with nicotine in human pregnancy was also highlighted.

*ANIMAL STUDIES*

*Acute data*

The $LD_{50}$ of nicotine in experimental animals varies widely. Some relevant oral toxicity data is presented below.

| Species | Oral $LD_{50}$ |
|---|---|
| Dog | 9.2 mg/kg |
| Mouse | 3.3 mg/kg |
| Rat | 50 mg/kg |

*Reproductive studies*

Administration of nicotine in animal reproductive studies is associated with impairment of reproduction, lower fetal viability, reduced reproductive potential, lower litter sizes and developmental delays.

*Developmental studies*

Administration of nicotine to monkeys during pregnancy results in changes to pulmonary function in their offspring, strikingly similar to the changes observed in offspring of human smokers, and alterations to known regulators of energy balance in the newborn offspring.

*Cardiovascular studies*

Administration of nicotine to squirrel monkeys results in the development of atherogenic blood lipid profiles and morphological changes in aortic endothelial tissue in New Zealand rabbits.

*Genotoxicity study*

Gross chromosomal aberrations including fuzzy chromosomes, aneuploidy and translocations were observed in mice receiving low tolerable doses of nicotine. Nicotine has demonstrated genotoxic activity at concentrations, comparable to saliva levels of nicotine achieved during tobacco chewing. However, in the Ames *Salmonella typhimurium* mutagenicity assay nicotine does not possess mutagenic activity, although it induced reparable DNA damage in *Escherichia coli* pol A+/A- system. The frequency of SCE's in the CHO system indicated that nicotine induced a weak positive result at very high concentrations.

*HUMAN STUDIES*

Human studies of nicotine presented in this safety assessment report primarily examine the tolerance and physiological effects of nicotine in smokers and non-smokers associated with nicotine replacement therapies and tobacco abatement.

Acceptable daily intake (ADI) and other guideline levels relevant to food safety have not been established for nicotine.

*Acute studies*

A number of poisonings and deaths have been reported in humans, primarily involving nicotine-containing pesticides. The mean lethal dose in human has not been adequately studied, however has been estimated to be 30-60 mg (0.5-1.0 mg/kg) in adults and is considered to be about 10 mg in children.

*Repeat dose studies*

Randomised, double-blind, placebo controlled inpatient/outpatient on habituated smokers administered nicotine by a transdermal patch showed that nicotine toxicity effects could result in light smokers assigned a high dose patch.

*Reproductive studies*

The safety and efficacy of nicotine replacement therapy (NRT) for smoking cessation during pregnancy have not been well studied. For the purposes of nicotine replacement therapy, nicotine is classified by the US Food and Drug Administration as a Pregnancy Category D drug - evidence of fetal risk, but benefits outweigh the risk.

*Cardiovascular Studies*

Transdermal administration of nicotine (21 mg/day) significantly increased heart rate compared with the control group without nicotine substitution.

Administration of nicotine (3 mg) by nasal spray to healthy non-smoking men and women resulted in a statistically significant increase in systolic blood pressure (7.1 ± 9.4% for the nicotine group vs. –1.6 ± 7.3% for the placebo group; P=0.03), but not diastolic blood pressure and heart rate.

Nicotine gum (4 mg) resulted in statistically significant increases in mean arterial blood pressure (+8 ± 1 mm Hg, P<0.001) and heart rate (+13 ± 3 beats/min, P<0.001) in non-smokers. In smokers, mean arterial pressure and heart rate increased similarly.

*Overall conclusion*

The safety assessment demonstrates developmental, reproductive and cardiovascular effects of nicotine administration in animal studies. Genotoxicity studies on nicotine indicate weakly positive activity. There is evidence of cardiovascular effects in human studies. Although identified as a potential risk factor, currently there are a lack of data with respect to the effect of nicotine on human pregnancy.

The available data indicates that there are significant safety concerns associated with the use of nicotine, however, currently there are insufficient data to establish a safe level of intake for nicotine.

## INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is primarily grown for the leaves. When cured, leaves are used for smoking, as cigars, cigarettes, or in pipes, or chewed, or used as snuff along with other ingredients. Tobacco dust was widely used on vegetable crops as an insecticide, or made into a liquid form, commonly known as black leaf 40. Medicinally the leaves have sedative, narcotic, emetic, antispasmodic activities and used for rheumatic swelling and skin diseases. As an oral medicinal agent, it produced great depression, emesis and convulsions, sometimes in very moderate doses and for this reason is rarely used (Duke 2001).

In the more than 60 species of *Nicotiana*, most alkaloids are 3-pyridyl derivatives with nicotine the principal alkaloid in 50 to 60% of species. Based on the amounts of alkaloid accumulation in leaves of *Nicotiana* species, nicotine, nornicotine, anatabine and anabasine are the major alkaloids present in the genus. There are many minor alkaloids found in tobacco leaves that are derivatives of the major alkaloids. Most of the minor alkaloids are present in less than 50 μg/g dry weight and many are present in nanogram amounts (Bush et. al., 1993).

The assessment of safety of *Nicotiana* species in food in this report has focussed primarily on the presence of nicotine. Nicotine in tobacco smoking concentrations, is a powerful psychoactive drug. Nicotine is the major cause of the predominant behavioural effects of tobacco and some of its physiological consequences. Human use of nicotine from tobacco meets the criteria for a drug of dependence (US Department of Health and Health Services 1988).

Nicotine is a tertiary amide consisting of a pyridine and a pyrrolidine ring (Figure 1). Stereochemistry is an important issue. Nicotine has one asymmetric centre and, as a result, exists as a pair of optical isomers (enantiomers). Nicotine in tobacco is largely, if not entirely, the laevorotatory (S)-isomer. Small amounts of the (R)-isomer (up to 5% of the total nicotine) are found in tobacco smoke, presumably formed by racemisation during combustion (Pool et. al., 1985). Pharmacological studies in animals and with *in vitro* preparation have shown that the (S)-isomer is more potent, with potencies of five to 100 times that of the (R)-isomer, depending on the system. In addition, it is known that the enantiomers are metabolised differently (Jacob III and Benowitz 1993).

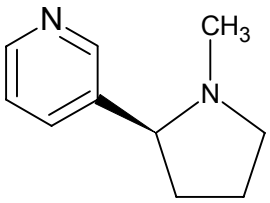



**Figure 1. Chemical structure of (S)-nicotine**

Every part of the tobacco plant except the seed contains nicotine. The concentration is related to different factors such as species, type of land, culture or weather conditions. The concentration of nicotine increases with the age of the plant. Tobacco leaves contain 2 to 8% of nicotine as the malate or citrate salt (de Landoni 1991a). The distribution of the nicotine in the mature plant is widely variable with high concentrations present in the leaves, though nicotine is also present to a lesser extent in the stem, root and flower of the tobacco plant.

**Absorption, Distribution, Metabolism and Excretion (ADME)**

*Absorption*

Nicotine is a water and lipid soluble drug which, in the free base form, is readily absorbed via respiratory tissues, skin, and the gastrointestinal tract (de Landoni 1991b). Absorption of nicotine across biological membranes depends on pH (Armitage and Turner 1970 ; Schievebein et. al., 1973).

Nicotine is a weak base with a $pK_a$ of 8.0. This means at pH 8.0, 50 percent of nicotine is ionised and 50 percent is non-ionised. In its ionised state, such as in acidic environments, nicotine does not rapidly cross membranes (US Department of Health and Health Services 1988). Nicotine is poorly absorbed from the stomach due to the acidity of gastric fluid (Travell 1960), but is well absorbed in the small intestine, which has a more alkaline pH and a large surface area (Jenner et. al., 1973).

About 69 percent of nicotine is ionised and 31 percent non-ionised after nicotine is absorbed into the blood (pH 7.4). Binding to plasma proteins is less than 5 percent (Benowitz et. al., 1982).

The intestinal bioavailability of nicotine and associated 'first-pass' metabolism suggests the bioavailability of orally administered nicotine is low (approximately 20%) (Zins et. al., 1997). This contrasts with the high absorption of nicotine from cigarette smoke (approximately 90%) (Gabrielsson and Gumbleton 1993).

*Distribution*

Nicotine is rapidly and extensively distributed throughout the body. Thirty to 60 minutes after intravenous administration of nicotine to rats, nicotine concentrations, two to 15 times higher than those in plasma have been observed in a number of organs, i.e. adrenals, liver, brain, lung, heart, gastro-intestinal tissue, spleen, thymus and kidney in addition to skeletal muscle (Gabrielsson and Gumbleton 1993).

Nicotine and the primary metabolite of nicotine, cotinine, are transferred to the human fetus, placenta and amniotic fluid of smoking mothers. Nicotine concentrations in the placenta (range 3.3-28 ng/g), in amniotic fluid (range 1.5-23 ng/ml) and in fetal serum (range 0.5-25 ng/ml) were all higher than the corresponding maternal serum values, while cotinine concentrations in placental tissue (range 10-131 ng/g), amniotic fluid (range (5-188 ng/ml) and fetal serum (range 15-233 ng/ml) were lower than or similar to corresponding maternal serum levels (Luck et. al., 1985).

A wide range of nicotine concentrations (<20-512 ng/ml) have been demonstrated in the milk of nursing mothers (Luck and Nau 1987). There is a linear correlation between both nicotine and cotinine concentrations in serum (or plasma) and milk of nursing mothers. Nicotine has been found to concentrate in breast milk, with milk:serum concentration ratios averaging 2.9 in a group of nursing mothers; the nursed infants serum:maternal serum nicotine concentration ratio averaging 0.06 (Luck and Nau 1984). Heavy smoking (20-30 cigarettes per day) may alter the supply of milk and cause nausea and vomiting in the infant (de Landoni 1991b).

High concentrations of nicotine have observed in the salivary gland, the concentration ratio of nicotine in saliva:plasma generally exceeds 10 (Russell and Feyerabend 1978). Passage of saliva containing nicotine into the stomach, combined with the trapping of nicotine in the acidic gastric fluid and re-absorption from the small bowel, provides a potential route for enteric nicotine recirculation. This recirculation may account for some of the oscillations in the terminal decline phase of nicotine in blood levels after i.v. nicotine infusion or cessation of smoking (de Landoni 1991b).

*Metabolism and Excretion*

Nicotine is rapidly and extensively metabolised, primarily in the liver, but also to a smaller extent, in the lung. The proximate metabolite is cotinine, which accounts for, on average, 80-90% of nicotine metabolism (Benowitz et. al., 1994). Cotinine is, in turn, metabolised to *trans*-3'-hydroxycotinine, the latter of which is the most abundant nicotine metabolite in the urine (Benowitz 1998).

Cotinine has a longer elimination half-life than nicotine (15 h compared to 2 h) (Curvall and Kazemi Vala 1993) although there is considerable individual variability. Regular nicotine exposure results in average plasma levels of cotinine about 15 times those of nicotine. Plasma levels of *trans*-3'-hydroxycotinine are about three times higher than those of nicotine (Benowitz et. al., 1997).

The kidney is the major organ for excretion of nicotine in man and other mammals. Secretion of nicotine into the bile contributes relatively insignificantly to overall clearance (Turner 1969).

The renal excretion of nicotine displays pH dependency; at a urinary pH above 7, nicotine is readily reabsorbed through passive diffusional transfer across the renal tubule back into the renal tubular circulation, with the result that as little as 2% of a dose is excreted unchanged in the urine. When urine is more acidic (pH less than 5), as much as 23% of the dose can be recovered in the urine (Gabrielsson and Gumbleton 1993).

Studies have shown that the main metabolites of nicotine found in urine after intravenous administration of nicotine to humans, and in the urine of tobacco users, are cotinine, trans-3'-hydroxycotinine, glucuronic acid derivatives of nicotine, cotinine and 3-hydroxycotinine, nicotine-1'-N-oxide, cotinine-1-N-oxide, nornicotine, norcotine and N-methyl-nicotinium ions (Curvall and Kazemi Vala 1993).

## ANIMAL STUDIES

### Acute toxicity studies

In experimental animals, the $LD_{50}$ of nicotine varies widely, depending on the route of administration and the species used. Intravenous injections result in the highest blood and brain concentrations and produce toxicity at the lowest doses while, with oral or intraperitoneal administration, higher doses are required to produce toxicity. This is due to pre-systemic ('first pass') metabolism of nicotine and the gradual time course of absorption as compared with intravenous dosing. With intermittent dosing, such as practiced by smokers, the total dose of nicotine absorbed per day could exceed the toxic or even lethal dose of a single injection (US Department of Health and Health Services 1988).

Relevant animal data associated with acute toxicity of orally administration nicotine are present in below (NIOSH 1996).

| Species | Oral $LD_{50}$ |
|---|---|
| Dog | 9.2 mg/kg |
| Mouse | 3.3 mg/kg |
| Rat | 50 mg/kg |

**Reproductive studies**

**Nicotine Injection during gestation: impairment of reproduction, fetal viability, and development** (Hudson and Timiras 1972).

| | |
|---|---|
| Test material: | Nicotine |
| Control group: | Saline (0.9%) |
| Test Species: | Long-Evans rats |
| Administration: | Subcutaneous injection twice daily |
| Series I: | Duration 0-21 day and terminated in all animals when the first set delivered. |
| Dose I: | 0 (0.9% saline), 1,3, and 5 mg/kg per injection (total daily dose of 2,6, and 10 mg/kg) |
| Series II: | Duration 0-7 day gestation, and allowed to deliver spontaneously. |
| Dose II | 0 (0.9% saline) or 3 mg/kg nicotine |

*Study Conduct*

The number per group varied from 4 to 13. Maternal weight gain, length of pregnancy, and birth weight of offspring were determined for those delivering. By C-section, the number of fetuses, crown-rump length, placental weight and number of corpora lutea were recorded.

*Results*

Nicotine effects were similar for both series: a dose related decline in reproductive capacity and an increase in resorptions were observed with increasing dose. Maternal mortality was increased (36% and 38% at 3 and 5 mg/kg, respectively) and the number of viable litters decreased, body weight of fetuses by C-section and maternal body weight gain were reduced in the mid and high dose groups. New born body weights were comparable but gestation length was statistically significantly longer at 3 and 5 mg/kg/dose. The percentage of implants was not affected but fewer survived.

**The effect of nicotine and alcohol on the fertility and life span of rats, a cytological analysis** (Riesenfeld and Oliva 1987).

| | |
|---|---|
| Test material: | Nicotine |
| Test species: | Female Fisher rats (96); female Buffalo rats (46) |
| Administration | Intramuscular injection, three times daily |
| Dose: | 0.42 mg/kg |

*Study conduct*

Dosing was initiated at 50 days of age, before the onset of puberty with female Fisher rats (96) and female Buffalo rats (46). Injection sites were rotated (interrupted for 30 days during nursing)

*Results*

50% of the Fisher and 49.7% of the Buffalo rats were sterile, after treatment with nicotine. This compares with a historical rate of 21%. The life span of both nicotine treated maternal rat species was significantly shortened (100 days versus 502 of the Fisher and 131 versus 472 for Buffalo rats). The age at first parturition was comparable for the Fisher rats (84 versus 86 for controls) but the last parturition was statistically lower (175 versus 386 days of age). For Buffalo rats, the first parturition was delayed on average by 21 days (105.4 versus 84.5 for controls) and the last was also earlier (175 versus 465 for controls. The number of neonates for Fisher rats was 76 for 48 injected dams and 21 for Buffalo rats. Offspring of treated dams that were fetally and postnatally exposed to nicotine either failed to give birth or had offspring that died shortly after birth, becoming 'extinct' after one generation. Cytology revealed a mild increase in lymphocytes and/or polymorphonuclear leukocytes in nicotine treated rats, appearing much earlier in Buffalo than Fisher rats and considered associated with inflammation. Offspring (untreated themselves) were normal as were offspring born after treatment of dams ceased, the inflammation seeming to be reversible.

**Nicotine reduces embryo growth, delays implantation and retards parturition in rats** (Hammer and Mitchell 1979).

| | |
|---|---|
| Test material: | Nicotine (98%) |
| Test species: | Female Sprague-Dawley rats |
| Administration | Subcutaneous injection, twice daily during post-coitum days 0 through 5 to mated rats |
| Dose: | 5 mg/kg |

*Study conduct*

Two test series were used, one to evaluate embryo growth and the other to determine effects of nicotine on fecundity and time of parturition. For effects on implantation, uterine horns were flushed with saline at selected times and the blastocysts retrieved.

*Results*

Loss of zona pellucida was slower and cell proliferation was significantly delayed for nicotine treated rats, but the size, weight, sex, or mortality of the offspring was not significantly affected.

**Effect of nicotine on the development of fetal and suckling rats** (Hamosh et. al., 1979).

| | |
|---|---|
| Test material: | Nicotine (98%) |
| Control: | 0 (untreated) and 0 (sterile saline by injection) |
| Test species: | Female Sprague-Dawley rats |
| Administration | Subcutaneous injection, three times daily (⅓ of total each time at a different site) and/or osmotic minipump (to alleviate the stress of injections) to mated rats |
| Dose: | 100 μg/kg/day or 1 mg/kg/day |
| Group size: | 6-11 |

*Study conduct*

Dosing began on day 14 of gestation and continued throughout the study. Fetuses were collected on day 20 of gestation and weight, length measured. Others were allowed to deliver normally. Suckling rats were sacrificed at several ages for examination of length, weight and stomach contents for lipid content and free fatty acids.

*Results*

Litter size was reduced in the higher dose group, being 8.8 versus 10.0 in controls, with 6 stillborn to 5 dams versus 1/12 litter in controls. The development of pups from nicotine treated dams (100 μg/kg/day) appeared normal at birth and up to one week after birth, but thereafter became slower in terms of weight and length. Stomach contents were smaller in pups of nicotine-treated dams. Fat content in mg was lower at day 12 in pups of treated dams (100 μg/kg/day) and lipolytic activity was slower to increase, reaching normal levels by day 7. Nicotine interference with milk production was suggested.

**Overall conclusion**

Administration of nicotine in animal reproductive studies is associated with impairment of reproduction, lower fetal viability, reduced reproductive potential, lower litter sizes and developmental delays.

**Developmental studies**

**Prenatal nicotine exposure alters pulmonary function in newborn rhesus monkeys** (Sekhon et. al., 2001).

| | |
|---|---|
| Test material: | Nicotine |
| Control: | Saline |
| Test species: | Rhesus monkey (two groups of 7 animals) |
| Administration | Subcutaneous osmotic pump |
| Dose: | 1.5 mg/kg/day |
| Duration: | day 26 through 160 of gestation (term = 165 days) |
| Group: | n=7 |

*Study conduct*

Timed pregnant rhesus monkeys were infused with either nicotine or saline from Day 26 to 160 of gestation. On Day 160 of pregnancy (term = 165 d), fetuses were delivered by C-section, and the following day were subjected to pulmonary function testing. After testing, animals were sacrificed, and lungs weighed and fixed.

*Result and conclusion*

Lung weight and fixed lung volume decreased (16% and 14%, respectively) significantly following *in utero* nicotine exposure. Peak tidal expiratory flow, FEV(0.2), mean mid-expiratory flow, forced expiratory volume at peak expiratory flow (FEV(PEF)), and FEV(PEF)/FVC% were significantly lower in newborns exposed to nicotine during gestation. Absolute and specific pulmonary resistance increased significantly whereas absolute and specific dynamic compliance remained unchanged in prenatally nicotine-treated pups. These changes in pulmonary function are strikingly similar to the changes observed in offspring of human smokers and suggest a role for nicotine in the altered pulmonary mechanics observed in human infants whose mothers smoked during pregnancy.

**Chronic maternal nicotine exposure alters neuronal systems in the arcuate nucleus that regulate feeding behaviour in the newborn rhesus macaque** (Grove et. al., 2001).

| | |
|---|---|
| Test material: | Nicotine |
| Control: | Saline |
| Test species: | Rhesus monkey (two groups of 7 animals) |
| Administration | Subcutaneous osmotic pump |
| Dose: | 1.5 mg/kg/day |
| Duration: | day 26 through 160 of gestation (term = 165 days) |
| Group: | n=7 |

*Study conduct*

Pregnant rhesus monkeys were treated with nicotine tartrate (1.5 mg/kg x d) starting on d 26 of pregnancy and maintained through d 160 of gestation.

*Result*

Nicotine exposure had no significant effect on absolute birth weights of the neonatal monkeys, although there was a 10% reduction in birth weights with nicotine exposure when they were normalised to maternal weight. Postnatal d 1 plasma leptin levels were significantly reduced by about 50% in the nicotine treatment group compared with saline controls, suggesting that the infant monkeys exposed to nicotine may also have lower body fat levels. *In situ* hybridization studies demonstrated that chronic nicotine exposure resulted in a significant decrease in arcuate NPY mRNA (neuroprotein Y) expression in the neonatal monkeys. In addition, there was a 2-fold increase in POMC mRNA (Pro-Opiomelanocortin) in the arcuate nucleus in the nicotine-exposed group. These data suggest chronic maternal nicotine treatment alters levels of known regulators of energy balance in the newborn offspring.

**Overall conclusion**

Administration of nicotine to monkeys during pregnancy results in changes to pulmonary function in their offspring, strikingly similar to the changes observed in offspring of human smokers, and alteration to known regulators of energy balance in the newborn offspring.

**Long-term toxicity studies**

**Oral nicotine induces an atherogenic lipoprotein profile** (Cluette-Brown et. al., 1986).

| | |
|---|---|
| Test material: | Nicotine |
| Test species: | Male squirrel monkey |
| Administration: | Nicotine in liquid diet |
| Control: | Isocaloric liquid diet |
| Dose: | 0 and 6.0 mg/kg/day |
| Duration: | 24 months |
| Group: | Treatment = 9, control = 9 |

*Study conduct*

Animals were weighed biweekly, plasma lipid, glucose, and lipoprotein parameters were measured monthly, and detailed lipoprotein composition, along with postheparin plasma lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) activity, was assessed after 24 months of treatment.

*Results and conclusion*

Although nicotine had no effect on plasma triglyceride or high density lipoproteins (HDL), the alkaloid caused a significant increase in plasma glucose, cholesterol, and low density lipoprotein (LDL) cholesterol plus protein while simultaneously reducing the HDL cholesterol/plasma cholesterol ratio and animal body weight. Levels of LDL precursors, very low density (VLDL) and intermediate density (IDL) lipoproteins, were also lower in nicotine-treated primates while total postheparin lipase (LPL + HTGL) activity was significantly elevated. The data indicate that long-term consumption of oral nicotine induces an atherogenic lipoprotein profile (increases LDL, decreases HDL/total cholesterol ratio) by enhancing lipolytic conversion of VLDL to LDL.

**Oral nicotine impairs clearance of plasma low density lipoproteins** (Hojnacki et. al., 1986).

| | |
|---|---|
| Test material: | Nicotine |
| Test species: | Male squirrel monkey |
| Administration: | Nicotine in liquid diet |
| Control: | Isocaloric liquid diet |
| Dose: | 0 and 6.0 mg/kg/day |
| Duration: | 24 months |
| Group: | Treatment = 9, control = 9 |
| Treatment: | $^{3}$H LDL and $^{14}$C high density lipoprotein (HDL) cholesteryl ester (CE) |

*Result and conclusion*

Averaged over 24 months of treatment, animals in the Nicotine group had significantly higher levels of plasma and LDL cholesterol compared to Controls while plasma LCAT activity was similar for both groups. Following simultaneous injection of $^{3}H$ LDL and $^{14}C$ high density lipoprotein (HDL) cholesteryl ester (CE), removal of the latter was not altered by oral nicotine while plasma clearance of $^{3}H$ LDL was dramatically delayed in Nicotine monkeys. Transfer of $^{14}C$ HDL CE to very low density lipoprotein (VLDL)-LDL particles was greatly accelerated in the Nicotine group vs. Controls while the reciprocal movement of $^{3}H$ LDL CE to HDL was only higher in experimental animals at two time points following injection of the isotopes. Results from this study provide evidence that one major detrimental effect of long-term oral nicotine use is an increase in the circulating pool of atherogenic LDL which is due to: 1) accelerated transfer of lipid from HDL; and 2) impaired clearance of LDL from the plasma compartment. Diminished removal of LDL is of particular importance because an extended residence time of these particles in circulation would increase the likelihood of their deposition in the arterial wall.

**Effects of chronic oral consumption of nicotine on the rabbit aortic endothelium** (Booyse et. al., 1981).

| | |
|---|---|
| Test material: | Nicotine |
| Test species (*in vitro*): | New Zealand white rabbit |
| Administration: | Nicotine in drinking water |
| Dose: | 0 and 2.4 mg/kg |
| Duration: | 25 weeks |
| Group: | Treatment = 10, control = 10 |

*Study conduct*

Nicotine-treated rabbits were compared with control rabbits in terms of blood serum biochemistry and lipid profiles, blood cells counts, changes in aortic endothelial cell morphologic characteristic and distribution, and vessel wall permeability (Evans blue dye uptake). Fasting serum levels of glucose, triglycerides, total cholesterol, and LDL-cholesterol were elevated in nicotine-treated rabbits.

*Result and conclusion*

No significant differences (nicotine vs. control) were seen in leukocyte, erythrocyte and platelet counts, or hematocrit and haemoglobin. Control and nicotine-treated rabbit aortas showed similar focal areas of increased Evans blue dye uptake; staining was localised primarily to aortic arch areas.

Endothelial cells (luminal surface) from non-Evans blue and Evans blue arch areas were examined by a combination of Hautchen preparation (silver-stained vessels) and scanning and transmission electron microscopy. Endothelial cells from nicotine-treated arch areas (Evans-blue-stained) showed extensive changes such as: increased cytoplasmic silver deposition, increased formation of microvilli, and numerous focal areas of 'ruffled' endothelium (projections on cell surfaces). These data indicate that nicotine, administered orally to rabbits, has a demonstrable *in vivo* morphologic effect on endothelial cells in the aortic arch.

**Overall conclusion**

Administration of nicotine to squirrel monkeys results in the development of atherogenic blood lipid profiles and morphological changes in aortic endothelial tissue in New Zealand rabbits.

## GENOTOXICITY STUDIES

**Mutagenicity testing, in bacterial systems, of some constituents of tobacco** (Riebe et. al., 1982).

*Test article*

Twelve constituents of tobacco including nicotine (1-20 mM) were tested in the Ames assay with *Salmonella typhimurium* and with *Escherichia coli* $polA^+/polA^-$ strains.

*Study conduct and result*

Salmonella strains were TA98, TA100 and TA1537, tested with and without activation using a pre-incubation of 1 hour before plating. There were three trials with triplicates. With *E. coli*, the diameter of growth inhibition using the spot test (in triplicate, two trials) and survival in liquid culture were determined. Nicotine did not significantly induce increases in revertants in the *Salmonella* strains. Nicotine was positive with *E. coli*, giving a larger diameter of growth inhibition with $polA^-$ strain

*Conclusion*

There was no evidence of mutagenicity induced by nicotine in *Salmonella* strains, but not in *E. coli* strains.

**Assessment of genotoxicity of nicotine employing *in vitro* mammalian test systems.** (Trivedi et. al., 1990).

*Test articles*

Nicotine (free base) was tested with Chinese hamster ovary cells at concentrations of 625 and 1000 μg/ml (first experiment and at a concentration of 150, 250, 375, 500 and 650 μg/ml (second experiment) for genotoxicity without activation.

*Study conduct and results*

Both chromosomal aberration and sister chromatid exchanges (SCE) were evaluated. Experiment I, cells were exposed for 2 or 4 hours, washed, and BrdU added. For aberrations, cells were harvested after 24 hours and after 2 cell cycles for SCE. Experiment II, nicotine was present until harvest at 24 hours for aberration or 48 hours for SCE analysis, also with BrdU. For aberrations, 100 metaphases in MI were scored and 25 metaphases in MII for SCE.

After 2 or 4 hours (experiment I), aberrations were increased at 1000 μg/ml. SCE were statistically increased for both exposure times and concentrations.

In experiment II, nicotine induced chromosomal aberrations at concentrations of 375 μg/ml and higher with continuous exposure in increased SCE frequency at all tested concentrations (150 – 625 μg/ml).

*Conclusion*
SCE frequency was highly significant for all concentrations of nicotine, whereas, statistically significant elevation of chromosomal aberration was observed only by the highest concentrations. Nicotine induced SCE's in a dose dependent manner. Nicotine was genotoxic at concentrations, comparable to saliva levels of nicotine achieved during tobacco chewing.

**The *in vitro* and *in vivo* cytogenetic effects of nicotine** (Bishun et. al., 1972).

*Test articles*

Nicotine was evaluated for genotoxicity *in vitro* using human peripheral blood lymphocytes at concentrations of 0, 0.5, 1.0, 1.5, and 2.0 μg/ml with exposure starting when cultures were initiated. Nicotine was investigated for cytotoxicity *in vivo* using groups of 12 randomly bred mice, ages from 5 weeks to 4 months, were injected with doses of 0 (saline, 0.07, 0.08 and 0.09 μg/total body weight, two injections per week for three weeks prior to sacrifice.

*Study conduct and results*

*In vitro* incubation were 6, 24, 48 and 72 hours with a total incubation time of 72 hours for all treatments. Duplicate cultures. Bone marrow cells were analysed for aberrations in the *in vivo* study. Cytotoxicity precluded genotoxic evaluation in the *in vitro* test. Aneuploidy and translocations were observed *in vivo*.

*Conclusion*

Nicotine at concentrations of 0.5 μg/ml only becomes toxic in the human lymphocyte blood cultures after 72 hours. Cytotoxic effects of nicotine were observed *in vitro*, without producing any chromosome damage. Gross chromosomal aberrations including fuzzy chromosomes, aneuploidy and translocations were observed in mice receiving low tolerable doses of the drug.

**Studies on the induction of sister-chromatid exchanges in Chinese hamster ovary cells by various tobacco alkaloids** (Riebe and Westphal 1983).

*Test articles*

Five tobacco alkaloids (nicotine - 1250, 2500 and 5000 μg/ml, myosine, anabasine, anatabine and nornicotine) were tested with and without S9 rat liver microsomal metabolic activation for genotoxicity using Chinese hamster ovary (CHO) cells. PBS and DMSO were negative controls.

*Study conduct and result*

Cell cultures were treated for 1 hour, BrdU added and incubation continued for 42 h. the assay was repeated in triplicate. Between 30 and 140 metaphases per concentration were for SCE. Nicotine induced a very slight increase in SCE rate of CHO cells. This effect was not enhanced by the use of metabolic activation.

*Conclusion*

The frequency of SCE's indicates that nicotine induced a weak positive response only at very high concentrations.

**Overall conclusion**

Gross chromosomal aberrations including fuzzy chromosomes, aneuploidy and translocations were observed in mice receiving low tolerable doses of the drug. Nicotine has demonstrated genotoxic activity at concentrations, comparable to saliva levels of nicotine achieved during tobacco chewing. However, in the Ames *Salmonella typhimurium* mutagenicity assay nicotine does not possess mutagenic activity, although it induced reparable DNA damage in *Escherichia coli* pol A+/A- system. The frequency of SCE's in the CHO system indicated that nicotine induced a weak positive result at very high concentrations.

**HUMAN STUDIES**

Human studies of nicotine presented in this safety assessment report primarily examine the tolerance and physiological effects of nicotine in smokers and non-smokers associated with nicotine replacement therapies and tobacco abatement.

**Acute toxicity**

In humans, acute exposure to nicotine even in low doses (similar to the amounts consumed by tobacco users) elicits autonomic and somatic reflex effects. Dizziness, nausea, and/or vomiting are commonly experience in non-smokers, after low doses of nicotine, such as when people try their first cigarette. However cigarette smokers rapidly become tolerant to these effects.

Nicotine intoxication produces nausea, vomiting, abdominal pain, diarrhoea, headaches, sweating and pallor. More severe intoxication results in dizziness, weakness, and confusion, progressing to convulsions, hypotension, and coma. Death is usually due to paralysis of respiratory muscles and or/central respiratory failure (US Department of Health and Health Services 1988).

A number of poisonings and deaths have been reported in humans, primarily involving nicotine-containing pesticides. The mean lethal dose in human has not been adequately studied, however has been estimated to be 30-60 mg (0.5-1.0 mg/kg) (Gosselin 1988) in adults and is considered to be about 10 mg in children (de Landoni 1991b).

**Short-term toxicity**

**High-dose nicotine patch therapy. Percentage of replacement and smoking cessation** (Dale et. al., 1995)

| | |
|---|---|
| Test materials: | Transdermal nicotine |
| Test species : | Human |
| Dose : | placebo, 11-, 22-, 44 mg/day |
| Group: | 71 healthy smokers stratified according to light (n =23), moderate (n = 24) and heavy (n = 24) |
| Duration: | 6-day inpatient stay, 7-week outpatient study |

*Study conduct*

Randomised, double-blind, placebo-controlled inpatient/outpatient trial. After baseline measures were obtained, subjects were randomly assigned to placebo or an 11-, 22-, or 44-mg/d dose of transdermal nicotine and admitted to a special hospital unit for intensive inpatient treatment of nicotine dependence. During the 6-day inpatient stay, daily nicotine and cotinine levels were determined from trough and peak blood samples. Outpatient patch therapy continued for 7 weeks following the hospital stay, and those initially assigned to placebo were randomly assigned to 11 or 22 mg/d. At week 4, the dosage of those initially assigned to 44 mg/d was reduced to 22 mg/d.

*Result and Conclusion*

A dose-response relationship was observed with higher patch doses, which produced a higher percentage of cotinine replacement and better withdrawal symptom relief. One subject (a light smoker assigned to the 44-mg dose) developed signs of nicotine toxicity.

**Studies on human pregnancy**

Cigarette smoking during pregnancy is the single largest modifiable risk for pregnancy-related morbidity and mortality in the US. Addiction to nicotine prevents many pregnant women who wish to quit smoking from doing so (Benowitz 1996). It is assumed that the cardiovascular effects of nicotine resulting in reduced blood flow to the placenta (uteroplacental insufficiency) is the predominant mechanism of the reproductive toxicity of cigarette smoking during pregnancy.

The safety and efficacy of nicotine replacement therapy (NRT) for smoking cessation during pregnancy have not been well studied. Nicotine for the purposes of nicotine replacement therapy, is classified by the US Food and Drug Administration as a Pregnancy Category D drug - risk of fetal injury, benefits outweigh the risks (Dempsey and Benowitz 2001).

**Studies on cardiovascular system**

**Transdermal nicotine increases heart rate after endotracheal intubation** (Puura 2003).

| | |
|---|---|
| Test materials: | Transdermal nicotine |
| Test species : | Human |
| Control: | 11 aged matched non-smokers |
| Dose : | placebo, 21 mg/day |
| Group: | 60 healthy smokers |
| Duration: | Treatment began at least 10h before anaesthesia. |

*Study conduct*

After induction with standardised doses of fentanyl, thiopental and atracurium, the patients were intubated immediately when EMG-response decreased to 10% of the initial control. Heart rate and non-invasive arterial pressures were recorded 1 min and 5 min after intubation.

*Result and conclusion*

The transdermal nicotine system significantly increased heart rate compared with the control group without nicotine substitution.
Since the transdermal nicotine system increases heart rate it should not be used if tachycardia is potentially dangerous, such as in patients with ischemic heart disease.

**Pharmacokinetics and pharmacodynamic effects of nicotine nasal spray devices on cardiovascular and pulmonary function** (Fishbein et. al., 2000).

| | |
|---|---|
| Test materials: | Nicotine |
| Test species : | Human |
| Administration: | Nasal spray |
| Dose: | 3 mg |
| Study type: | Double blind |
| Group: | 20 healthy, non-smoking men and women |

*Study conduct*

In this double-blind, randomised study of Nicotrol NS versus placebo, we measured serum nicotine concentrations, blood pressure, heart rate, and indices of pulmonary function at timed intervals before and after nasal spray administration of 3 mg of nicotine.

*Result and conclusion*

A peak serum nicotine concentration of 4.71 +/- 3.16 ng/mL occurred 10 minutes after drug administration. The maximum change in systolic blood pressure occurred 5 minutes after dosing and was significantly related to nicotine administration (7.1 +/- 9.4% for the nicotine group vs. -1.6 +/- 7.3% for the placebo; P = 0.03). In contrast, neither diastolic blood pressure (P = 0.8) nor heart rate (P = 0.07) changed significantly after nicotine administration, when compared with placebo. Pulmonary function was not altered acutely by a single inhalation of nicotine.

Pharmacokinetic modelling revealed a classic one-compartment model in which nicotine is absorbed into the systemic circulation by a zero-order process and eliminated by a first-order process. In this population of non-smokers, haemodynamic effects of the nicotine nasal spray were observed shortly after administration and before the peak serum nicotine concentration.

**Contrasting renal effects of nicotine in smokers and non-smokers** (Halimi et. al., 1998).

| | |
|---|---|
| Test materials: | Nicotine |
| Test species : | Human |
| Administration: | Nicotine gum |
| Dose : | 4 mg |
| Group: | non-smokers and chronic smokers |

*Study conduct*

The acute effects of a 4-mg nicotine gum on arterial pressure, heart rate as well as renal haemodynamics and function were assessed in non-smokers and chronic smokers.

*Results and conclusion*

In non-smokers, mean arterial pressure (+8 +/- 1 mmHg, $P<0.001$) and heart rate (+13 +/- 3 beats/min, $P<0.001$) increased whereas effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) decreased by 15 +/- 4% and 14 +/- 4% respectively; in addition, urinary cyclic GMP decreased by 51 +/- 12% in response to nicotine administration.

In smokers, mean arterial pressure and heart rate increased similarly; however, in contrast with non-smokers, ERPF and GFR remained unchanged whereas urinary cyclic GMP rose by 87 +/- 43%. Changes in ERPF induced by nicotine were positively correlated with changes in urinary cyclic GMP. These findings indicate that nicotine administration is associated with renal vasoconstriction in healthy non-smokers, possibly through alteration of a cyclic-GMP-dependent vasoactive mechanism.

**Overall conclusion**

The safety assessment demonstrates developmental, reproductive and cardiovascular effects of nicotine administration in animal studies. Genotoxicity studies on nicotine indicate weakly positive activity. There is evidence of cardiovascular effects in human studies. Although identified as a potential risk factor, currently there are a lack of data with respect to the effect of nicotine on human pregnancy.

The available data indicates that there are significant safety concerns associated with the use of nicotine, however, currently there are insufficient data to establish a safe level of intake for nicotine.

Reference List


Andersson, C., Wennström, P., and Gry, J. (2003). Nicotine alkaloids in Solanaceous food plants. *TemaNord* 531: 1-37.

Armitage, A.K. and Turner, D.M. (1970). Absorption of nicotine in cigarette and cigar smoke. *Nature* 226 (5252): 1231-1232.

Benowitz, N.L. (1996). Pharmacology of nicotine: Addiction and therapeutics. *Annu.Rev.Pharmacol.Toxicol.* 36: 597-613.

Benowitz, N L (1998). Cardiovascular Toxicity of Nicotine: Pharmacokinetic and Pharmacodynamic Considerations. in *Nicotine Safety and Toxicity*. Edited by Benowitz,N.L. Published by Oxford University Press. New York. Chapter 2, 19-27.

Benowitz, N.L., Jacob, P.I., Fong, I., and Gupta, S. (1994). Nicotine metabolic profile in man: Comparison of cigarette smoke and transdermal nicotine. *Journal of Pharmacology and Experimental Therapeutics* 268: 296-303.

Benowitz, N.L., Jacob, P.I., Jones, R.T., and Rosenberg, J. (1982). Interindividual variability in the metabolism and cardiovascular effects of nicotine in man. *Journal of Pharmacology and Experimental Therapeutics* 221 (2): 368-372.

Benowitz, N.L., Zevin, S., and Jacob III, P. (1997). Sources of variability in nicotine and cotinine levels with use of nicotine nasal spray, transdermal nicotine, and cigarette smoking. *Br.J.Clin.Pharmacol.* 61: 259-267.

Bishun, N.P., Lloyd, N., Raven, R.W., and Williams, D.C. (1972). The *in vitro* and *in vivo* cytogenetic effects of nicotine. *Acta Biologica Academiae Scientiarum Hungaricae* 23 (2): 175-180.

Booyse, F.M., Osikowicz, G., and Quarfoot, A.J. (1981). Effects of chronic oral consumption of nicotine on the rabbit aortic endothelium. *American Journal of Pathology* 102 (2): 229-238.

Bush, L P, Fannin,F.F., Chelvarajan,R.L., and Burton,H.R. (1993). Biosynthesis and metabolism of nicotine and related alkaloids. in *Nicotine and Related Alkaloids: Absorption, distribution, metabolism and excretion.* Edited by Gorrod,J.W. and Wahren,J. Published by Chapman & Hall. London. Chapter 1, 1-30.

Castro, A. and Monji, N. (1986). Dietary nicotine and its significance in studies on tobacco smoking. *Biomed.Arch.* 2 (2): 91-97.

Cluette-Brown, J., Mulligan, J., Doyle, K., Hagan, S., Osmolski, T., and Hojnacki, J. (1986). Oral nicotine induces an atherogenic lipoprotein profile. *Proc Soc.Exp.Biol.Med* 182 (3): 409-413.

Curvall, M and Kazemi Vala,E. (1993). Nicotine and metabolites: analysis and levels in body fluids. in *Nicotine and related Alkaloids: Absorption, distribution, metabolism and excretion.* Edited by Gorrod,J.W. and Wahren,J. Published by Chapman & Hall. London. Chapter 8, 147-179.

Dale, L.C., Hurt, R.D., Offord, K.P., Lawson, G.M., Croghan, I.T., and Schroeder, D.R. (1995). High-dose nicotine patch therapy. Percentage of replacement and smoking cessation. *JAMA* 274 (17): 1353-1358.

Davis, R.A., Stiles, M.F., de Bethizy, J.D., and Reynolds, J.H. (1991). Dietary intake: a source of urinary cotinine. *Food Chem.Toxicol.* 29 (12): 821-827.

de Landoni, J.H. (1991a). Poisons Information Monograph: *Nicotiana tabacum* L.

de Landoni, J.H. (1991b). Poisons Information Monograph: Nicotine.

Dempsey, D.A. and Benowitz, N.L. (2001). Risks and benefits of nicotine to aid smoking cessation in pregnancy. *Drug Saf* 24 (4): 277-322.

Domino, E.F., Hornbach, E., and Demana, T. (1993). Relevance of nicotine content of common vegetables to the identification of passive tobacco smoke. *Med.Sci.Res.* 21 (571): 572-

Duke, J. A. (2001). Handbook of Medicinal Herbs. Published by CRC Press LLC. Boca Raton FL.

Feckler, L.F., Rügenhagen, C., and Berlin, J. (1993). Increased production of cadaverine and anabasine in hairy root cultures of Nicotiana tabacum expressing bacterial lysine decarboxylase gene. *Plant Mol.Biol.* 23: 11-21.

Fishbein, L., O'Brien, P., Hutson, A., Theriaque, D., Stacpoole, P.W., and Flotte, T. (2000). Pharmacokinetics and pharmacodynamic effects of nicotine nasal spray devices on cardiovascular and pulmonary function. *J Investig.Med* 48 (6): 435-440.

Gabrielsson, J and Gumbleton,M. (1993). Kinetics of nicotine and its metabolites in animals. in *Nicotine and Related Alkaloids: Absorption, distribution, metabolism and excretion.* Edited by Gorrod,J.W. and Wahren,J. Published by Chapman & Hall. London. Chapter 9, 181-195.

Gosselin, R. E. (1988). Clinical toxicology of commercial products. 4th ed. Published by Williams & Wilkins. Baltimore.

Grove, K.L., Sekhon, H.S., Brogan, R.S., Keller, J.A., Smith, M.S., and Spindel, E.R. (2001). Chronic maternal nicotine exposure alters neuronal systems in the arcuate nucleus that regulate feeding behavior in the newborn rhesus macaque. *J Clin Endocrinol.Metab* 86 (11): 5420-5426.

Halimi, J.M., Philippon, C., and Mimran, A. (1998). Contrasting renal effects of nicotine in smokers and non-smokers. *Nephrol.Dial.Transplant.* 13 (4): 940-944.

Hamill, J.D., Robins, R.J., Parr, A.J., Evans, D.M., Furze, J.M., and Rhodes, M.J.C. (1990). Over-expressing a yeast ornithine decarboxylase gene in transgenic roots of *Nicotiana rustica* can lead to enhanced nicotine accumulation. *Plant Mol.Biol.* 15: 27-38.

Hammer, R.E. and Mitchell, J.A. (1979). Nicotine reduces embryo growth, delays implantation, and retards parturition in rats. *Proc Soc.Exp.Biol.Med* 162 (2): 333-336.

Hamosh, M., Simon, M.R., and Hamosh, P. (1979). Effect of nicotine on the development of fetal and suckling rats. *Biol.Neonate* 35 (5-6): 290-297.

Hojnacki, J., Mulligan, J., Cluette-Brown, J., Igoe, F., and Osmolski, T. (1986). Oral nicotine impairs clearance of plasma low density lipoproteins. *Proc Soc.Exp.Biol.Med* 182 (3): 414-418.

Holmberg, N., Lilius, G., Bailey, J.E., and Bulow, L. (1997). Transgenic tobacco expressing *Vitreoscilla* haemoglobin exhibits enhanced growth and altered metabolic production. *Nature Biotechnol.* 15: 244-247.

Hudson, D.B. and Timiras, P.S. (1972). Nicotine injection during gestation: impairment of reproduction, fetal viability, and development. *Biol.Reprod.* 7 (2): 247-253.

Jacob III, P and Benowitz,N.L. (1993). Pharmacokinetics of (S)-nicotine and metabolites in humans. in *Nicotine and Related Alkaloids: Absorption, distribution, metabolism and excretion.* Edited by Gorrod,J.W. and Wahren,J. Published by Chapman & Hall. London. Chapter 10, 197-218.

Jenner, P., Gorrod, J.W., and Beckett, A.H. (1973). The absorption of nicotine-1'-N-oxide and its reduction in the gastro-intestinal tract in man. *Xenobiotica* 3 (6): 341-349.

Luck, W. and Nau, H. (1984). Nicotine and cotinine concentration in serum and milk of nursing smokers. *Br.J.Clin.Pharmacol.* 18: 9-15.

Luck, W. and Nau, H. (1987). Nicotine and cotinine concentration in serum and milk of nursing mothers: influence of cigarette consumption and diurnal variation. *Eur.J.Pediatr.* 146: 21-26.

Luck, W., Nau, H., Hansen, R., and Steldinger, R. (1985). Extent of nicotine and cotinine transfer to the human fetus, placenta and amniotic fluid of smoking mothers. *Dev.Pharmacol.Ther.* 8 (6): 384-395.

NIOSH (1996). Documentation for immediately dangerous to life or health concentrations (IDLH's): Nicotine. NIOSH. Washington DC. http://www.cdc.gov.niosh/idhl/54115.html

Pool, W.F., Godin, C.S., and Crooks, P.A. (1985). Nicotine racemization during cigarette smoking. *The Toxicologist* 5: 232-

Puura, A. (2003). Transdermal nicotine increases heart rate after endotracheal intubation. *Methods Find.Exp.Clin Pharmacol.* 25 (5): 383-385.

Riebe, M. and Westphal, K. (1983). Studies on the induction of sister-chromatid exchanges in Chinese hamster ovary cells by various tobacco alkaloids. *Mutat.Res* 124 (3-4): 281-286.

Riebe, M., Westphal, K., and Fortnagel, P. (1982). Mutagenicity testing, in bacterial test systems, of some constituents of tobacco. *Mutat.Res* 101 (1): 39-43.

Riesenfeld, A. and Oliva, H. (1987). The effect of nicotine and alcohol on the fertility and life span of rats. A cytological analysis. *Acta Anat.(Basel)* 128 (1): 45-50.

Russell, M.A.H. and Feyerabend, C. (1978). Cigarette smoking: a dependence on high nicotine boli. *Drug Metab.Rev.* 8: 29-57.

Schievebein, H., Eberhardt, T., Loeschenkohl, K., Rahlfs, V., and Bedall, F.K. (1973). Absorption of nicotine through oral mucosa. I. Measurement of nicotine concentration in the blood after application of nicotine and total particulate matter. *Agents and Actions* 3 (4): 254-258.

Sekhon, H.S., Keller, J.A., Benowitz, N.L., and Spindel, E.R. (2001). Prenatal nicotine exposure alters pulmonary function in newborn rhesus monkeys. *Am J Respir Crit Care Med* 164 (6): 989-994.

Sheen, S.J. (1988). Detection of nicotine in foods and plant material. *J.Food Sci.* 53 (5): 1572-1573.

Siegmund, B., Leitner, E., and Pfannhauser, W. (1999). Determination of the nicotine content of various edible nightshades (Solanaceae) and their products and estimation of the associated dietary nicotine intake. *J.Agric.Food Chem.* 47 (8): 3113-3120.

Travell, J. (1960). Absorption of nicotine from various sites. *Annals of the New York Academy of Sciences* 90 (1): 13-30.

Trivedi, A.H., Dave, B.J., and Adhvaryu, S.G. (1990). Assessment of genotoxicity of nicotine employing in vitro mammalian test system. *Cancer Lett* 54 (1-2): 89-94.

Turner, D.M. (1969). Metabolism of [$^{14}$C] nicotine in the cat. *Biochem.J.* 115: 889-

US Department of Health and Health Services (1988). A Report of the Surgeon General, The Health Consequences of Smoking. Nicotine Addiction. US Department of Health and Health Services. Washington D.C.

Zins, B.J., Sandborn, W.J., Mays, D.C., Lawson, G.M., McKinney, J.A., Tremaine, W.J., Mahoney, D.W., Zinmeister, A.R., Hurt, R.D., Offord, K.P., and Lipsky, J.J. (1997). Pharmacokinetics of nicotine tartrate after single-dose liquid enema, oral, and intravenous administration. *J.Clin.Pharmacol.* 37 (5): 426-436.

## ATTACHMENT 3

# Summary of submissions

**Initial Assessment Report P278 – Use of Nicotine and *Nicotiana* Species in Food.**

A total of eleven submissions were received in response to the Initial Assessment Report (IAR), which was released for public comment in October 2003. Eight of the submissions were from Australia and three from New Zealand. Nine submissions strongly supported Regulatory Option 1 – Prohibit the use of *Nicotiana* species and all substances derived therefrom in all foods. One submission proposed a modified Regulatory Option 2 - Allow the use of *Nicotiana* species in all foods but restrict the level of nicotine to the level demonstrated to be safe and to not be therapeutic or psychoactive. One submission strongly recommends that FSANZ does not assume oversight for an area more appropriately the purview of tobacco control authorities.

**Woman's Christian Temperance Union (Sarah Mitchell)**

- Support Regulatory Option 1.
- Objects to nicotine being added to any product.

**Confectionery Manufacturers of Australasia (Jennifer Thompson)**

- Supports Regulatory Option 1.
- Does not believe that the inclusion of tobacco or substances derived from tobacco is appropriate in Standard 1.4.1 – Contaminants and Natural Toxicants.
- The levels of nicotine found naturally in various foods appear well known and Standard 1.4.1 should include maximum levels of nicotine in these foods.
- Where nicotine is added to products for the intention of medicated or therapeutic effects, the Therapeutic Goods Administration in Australia and the equivalent body in New Zealand should administer these products.

**Food Technology Association of Victoria (David Gill)**

- Support Regulatory Option 1.
- No perceived benefit in the use of tobacco or substances derived from tobacco in food.

**New Zealand Food Safety Authority (Carole Inkster)**

- Supports Regulatory Option 1.
- Does not support Regulatory Option 2, as they do not support the intentional addition of *Nicotiana* species, even at safe levels to food. This is because the NZFSA support the public health measures aimed at controlling tobacco use, and we do not wish to undermine this message by allowing the intentional addition of nicotine to foods.
- Where there is a product that has a pharmacological effect, the appropriate regulatory mechanism is under the Medicines Regulations.
- If a benefit is established for consuming a food derived from the *Nicotiana* species, this can be assessed for inclusion as a novel food. The issues of safe nicotine levels and any other toxic entities would need to be addressed.

**Crown Public Health (Janelle Mackie)**

- Supports Regulatory Option 1.
- Strongly opposes the other two options, and any other Regulatory Option that allows the addition of nicotine and *Nicotiana* species in food.
- There is no justifiable reason to allow the addition of nicotine to food.
- Highlights the potential negative impact on the overall social and environmental acceptance of nicotine if this was allowed in the food supply.

**Queensland Health (Kerry Bell)**

- Supports Regulatory Option 1.
- Supports government and non-government initiatives to improve the health of all Queenslanders by eliminating or reducing their exposure to tobacco in all its forms.
- Are concerned that the potential future uses of *Nicotiana* species in foods should be considered on a case-by-case basis.
- Clarifies that Queensland Health is opposed to the addition of nicotine to foods. Foods that naturally contain low levels of nicotine (e.g. vegetables) should not be affected by regulation as such, setting a tolerable level of nicotine requires careful consideration.

**Department of Human Services, South Australia (Garry Clarke)**

- Supports Regulatory Option 1.
- There does not appear to be much justification to warrant supporting the use of *Nicotiana* species in ordinary food.

**Heart Foundation (Susan Anderson)**

- Supports Regulatory Option 1.
- Does not support the addition of tobacco or nicotine to food.

**Ministry of Health (Dr Don Matheson)**

- Supports Regulatory Option 1.
- Agrees that the regulatory status of tobacco added to foods needs to be within the scope of the Food Standards Code.
- Does not support Regulatory Option 2, as it does not support the intentional addition of *Nicotiana* species to food, even at safe levels or at levels below and addictive threshold if this level can be determined. This is because the ministry supports the public health measures aimed at controlling tobacco use, and does not wish to undermine this message by allowing the intentional addition of nicotine to foods or to allow people to become addicted to, or maintain an addiction to nicotine, through the addition of nicotine to food.
- Considers that where there is a product that has a pharmacological effect, the appropriate regulatory mechanism is medicines regulation.

**British American Tobacco Australia Limited (Adam Bookless)**

- BATA does not oppose FSANZ's proposal to provide regulatory oversight for the use of nicotine and *Nicotiana* species in food, however wishes to ensure that in doing so FSANZ does not inadvertently inhibit the development of a potentially reduced exposure product intended as an alternative for smokers.
- BATA does not believe that FSANZ is the appropriate authority to oversee developments intended to provide a potentially reduced exposure alternative for smokers.
- BATA supports sensible regulatory measures by public health authorities to reduce the public health impact of tobacco products, which is believed to be consistent in principle with FSANZ's proposal to regulate nicotine in food.
- BATA strongly recommend that FSANZ does not assume oversight for an area more appropriately the purview of tobacco control authorities.

**VicHealth Centre for Tobacco Control (Dr Ron Borland)**

- Support a modified version of Regulatory Option 2 – Allow the use of *Nicotiana* species in all foods but restrict the level of nicotine to the level demonstrated to be safe and to not be therapeutic or psychoactive.
- Proposes that products that contain nicotine should not be allowed on the market as foods, where the level of nicotine is such that the products have therapeutic or psychoactive effects.
- Recognises that there are instances where products derived from *Nicotiana tabacum* L. demonstrate functionality appropriate for use in food.
- Does not advocate the banning of such foods, instead proposes a test of the therapeutic or psychoactive affect and safety.
- Views that the need for FSANZ to consider this review highlights the major problem with the current (tobacco) regulatory environment. States that a more effective and integrated regulation for nicotine-containing products that have therapeutic or psychoactive effects should be recommended in the FSANZ assessment.

## Summary of submissions

**Draft Assessment Report P278 – Use of Nicotine and *Nicotiana* Species in Food.**

A total of fourteen submissions were received in response to the Draft Assessment Report (DAR), which was released for public comment in March 2004. Ten of the submissions were from Australia and four from New Zealand. Twelve submissions strongly supported Regulatory Option 1 – Prohibit the use of *Nicotiana* species and all substances derived therefrom in all foods. Two submissions did not oppose the regulation of nicotine and *Nicotiana* species in food in principle, though one stated that consideration should be given to the use of tobacco as a biofactory and the other stated that consideration should be given to the development of potentially reduced exposure products ('PREPs') intended to be offered as an alternative to smokers.

Issues raised by four submitters have been addressed in section 6.2 of this Report are outlined below;

New Zealand Food Safety Authority (NZFSA) while supporting Option 1 noted that there is no clear statement in the Draft Assessment Report about the likelihood of nicotine being produced from sources other than *Nicotiana* species, for example synthetic sources or nicotine from other plant species. Such nicotine would not be prohibited by option 1 alone.

Cadbury Schweppes Pty Ltd while supporting Option 1, acknowledges that a number of food products where nicotine is present in natural forms and recommends that those foods are included in Standard 1.4.1, Clause 3 'Maximum levels of non-metal contaminants in food' along with a corresponding maximum level of permitted nicotine.

Department of Primary Industries and Fisheries (Queensland) appreciates that the use of tobacco and nicotine in food may promote or legitimise the smoking of tobacco or the use of smokeless tobacco products however highlights that tobacco is one of the prime candidates for use as a biofactory and therefore suggests that recommendations should allow legitimate opportunities to be considered and not be ruled out by a blanket recommendation.

British American Tobacco Australia (Limited) (BATA), which in principle does not oppose the regulation of the use of nicotine and *Nicotiana* species in food, believes that any proposed regulation in this regard, must give due consideration to, and make clear provision for developments of potentially reduced exposure products ('PREPs') intended to be offered as an alternative to smokers choosing to potentially minimise the risks associated with smoking.

**Confectionery Manufacturers of Australia Limited (CMA) (Deemy Dove)**

- Reiterates support to Regulatory Option 1.
- The CMA agrees with the concern expressed by health authorities as mentioned in the Draft Assessment Report, that the use of tobacco or nicotine in food may promote or legitimise the smoking of tobacco or the use of smokeless tobacco products.
- The CMA states that given there is insufficient data to establish a safe level for the intake of nicotine and its use in food is unjustifiable on public health and safety grounds.

**Australian Soft Drinks Association Ltd (ASDA) (Melanie McPherson)**

- Supports Regulatory Option 1.
- ASDA can see no value to customers to have such products available, nor do any members of ASDA wish to produce or see such products available on the market.

**Australasian Bottled Water Institute Inc. (ABWI) (Melanie McPherson)**

- Supports Regulatory Option 1.
- ABWI can see no value to customers to have such products available, nor do any members of ABWI wish to produce or see such products available on the market.

**Department of Primary Industries and Fisheries (Queensland) (Peter Tonello)**

- Appreciates the concern of health authorities that the use of tobacco or nicotine in food may promote or legitimise the smoking of tobacco or the use of smokeless tobacco products.
- Recommends that legitimate opportunities associated with the use of *Nicotiana* species as a biofactory should not be ruled out by a blanket recommendation.

**Cancer Society of New Zealand Inc. (Carolyn Watts)**

- Supports Regulatory Option 1.
- Recognises the public health and safety risks associated with the exposure to nicotine through smoking and use of smokeless tobacco products.
- Concerned that the use of tobacco or nicotine in foods may promote or legitimise the smoking of tobacco or use of smokeless tobacco products.

**National Council of Women of New Zealand – Te Kaunihera Wahine O Aotearoa (NCWNZ) (Lynda Sutherland)**

- Strongly supports Regulatory Option 1
- NCWNZ agrees that FSANZ would be the appropriate body to regulate the addition of nicotine and Nicotiana species to food.
- NCWNZ believes that if there is some therapeutic value in adding nicotine or other products from Nicotiana species to foods, the control and regulation should lie with the Medicines Control Authority.
- Strongly disagree with the submission from British American Tobacco (Australia) (BATA) whereby 'BATA strongly recommend that FSANZ does not assume oversight for an area more appropriately the purview of the tobacco control authorities.'
- Disagree with the comments from VicHealth Centre for Tobacco Control, specifically 'Support a modified version of Option 2 – Allow the use of Nicotiana species in all foods but restrict the level of nicotine to the level demonstrated to be safe and not to be therapeutic or psychoactive.'
- With respect to the VicHealth Centre for Tobacco Control, the NCWNZ raises the question of determining a safe level for children.

**Dietitians Association of Australia (DAA) (Ruth Kharis)**

- Support Regulatory Option 1.

**Department of Human Services SA (Food Section) (Joanne Cammans)**

- Support Regulatory Option 1.

**Environmental Health Unit of Queensland Health (Food Services) (Gary Bielby)**

- Strongly supports Regulatory Option 1.
- By supporting Option 1, Queensland Health believes Government is delivering a consistent tobacco control message to the general community and maintaining consumer confidence in the safety of our food supply.

**Cadbury Schweppes Pty Ltd (Neil Smith)**

- Supports Regulatory Option 1.
- Acknowledges a number of food products where nicotine is present in natural forms and recommends that those foods are included in Standard 1.4.1, Clause 3 – Maximum levels of non-metal contaminants in food – along with a corresponding maximum permitted level of nicotine.
- Cadbury Schweppes believe that by defining a maximum permitted level of nicotine in Standard 1.4.1, in those foods where nicotine occurs naturally, there is no risk that nicotine levels would be found in a mixed food at unacceptable levels.

**Food Technology Association of Victoria Inc. (FTA) (David Gill)**

- Supports Regulatory Option 1.

**New Zealand Food Safety Authority (NZFSA) (Carole Inkster)**

- Supports Regulatory Option 1.
- NZFSA does not support regulatory Option 2, as they do not support the intentional addition of Nicotiana species to foods, even at safe levels. This is because the NZFSA support the public health measures aimed at controlling tobacco use, and do not wish to undermine this message by allowing the intentional addition of nicotine to foods.
- Where there is a product that has a pharmacological effect, the appropriate regulatory mechanism is under Medicines legislation.
- NZFSA notes that there is no clear statement about the likelihood of nicotine being produced from sources other than *Nicotiana* species, for example synthetic sources or nicotine from other plant species, such nicotine would not be prohibited by option 1 alone.

**New Zealand Dietetic Association (NZDA) (Sandy Clementt)**

- Supports Regulatory Option 1.
- NZDA agrees there is insufficient data to establish a safe level of intake for nicotine in food and therefore it should not be considered to be a suitable additive in any quantity.
- NZDA supports the view that Option 1 maintains consumer confidence in the safety and regulation of the food supply and maintains the delivery of a consistent government tobacco control message to the general community.

**British American Tobacco Australia Limited (BATA) (Caroline Denyer)**

- BATA does not, in principle, oppose the regulation of the use of nicotine and *Nicotiana* species in food.
- BATA believes any proposed regulation in this regard, must give due consideration to, and make clear provision for, developments of potentially reduced exposure products (PREPs) intended to be offered as an alternative to smokers choosing to potentially minimise the risks associated with smoking.
- BATA defines PREPs as products that potentially result in a substantially reduced exposure to substances in tobacco smoke regarded as playing an important role in smoking and health.
- BATA believes FSANZ's recommendation to amend the Code to prohibit the use of nicotine in food will, in its current form, impede the development of any PREP which may be used orally.
- BATA believes the proposed regulation is outside the appropriate regulatory remit of FSANZ and encroaches on an area which is more appropriately dealt with by tobacco control authorities.
- BATA believes the proposed regulation will potentially undermine public health objectives to minimise the harm associated with tobacco use.
- BATA believes that if regulatory option 1 is to be pursued, it is imperative on public health grounds that specific provision be made for developments of potentially reduced exposure products intended to be offered as an alternative to smokers.
- BATA recommends that provision be made for products falling within FSANZ's definition of food, yet intended to offer a reduced exposure alternative to smokers, be referred to tobacco control authorities for review. Such a mechanism would allow tobacco control authorities to consider the issue in the intended context, rather than misappropriating the issue as one relating to food standards.