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A new rural industry – Stevia – to replace imported chemical sweeteners

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Foreword

This report presents background information supporting the decision to further invest in R & D to establish an industry around stevia. It brings together published literature and viewpoints of likely players in the new industry, and summarises the outcomes of visits to major producing and consuming countries in Asia. While further research and investigation is necessary there are potential economic, environmental and health benefits from the future development of this crop in Australia.

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Simon Hearn
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Executive Summary

Stevia is a plant with carbohydrate-based compounds that are 200-300 sweeter than sugar. These compound, steviosides, can be extracted and used as alternative sweeteners to sugars, of particular benefit to diabetics and those wishing to reduce sugar intake for health reasons.

A review of current literature, and visits to Japan and China were undertaken to determine the likely benefits or otherwise of establishing a stevia industry in Australia. While steviosides are not currently permitted as food additives in Australia, they are in China, Japan, Taiwan and Korea. During discussions with potential stakeholders in a stevia industry, it was determined to address the requirements by Food Standards Australia New Zealand (FSANZ – formerly ANZFA) for registration of stevia for use in the food industry, and to determine the economic feasibility of growing and processing stevia within Australia. These two sets of activity, which will be supported by industry, university and RIRDC funding, will draw heavily upon the literature review prepared, the contacts made during the overseas trips and on the reports that support use of steviosides in the aforementioned countries.

1. Introduction

Stevia rebaudiana is a small bush native to Paraguay. Its leaves contain approximately 10% of steviosides which are intensely sweet compounds (150 to 300 times sweeter than sugar). The leaves have been traditionally used for hundreds of years in Paraguay and Brazil to sweeten local teas, medicines and as a 'sweet treat'. Japan is now the largest consumer of steviosides extracted from stevia leaves; in Japan stevia replaces the chemical sweeteners, aspartame etc, which were banned there in the 1970's. Other countries use lesser quantities of steviosides.

Steviosides have zero calories and can be used wherever sugar is used, including in baking etc. They are ideal for diabetic and low calorie diets; in Japan 'diet Coke' uses steviosides.

The plant has been successfully grown under a wide range of conditions from its native sub-tropics to Thailand and Indonesia and the cold northern latitudes of Leningrad and north China and Canada. In cold climates it is grown over the summer period as a transplanted annual (like tobacco), with a single harvest. In tropical areas it is a perennial (2 to 5 years) and multiple harvests per year are possible. As an annual crop in Canada, it is suggested that 50 hectares of stevia could produce sweetener equivalent to $1 million of sugar which in Australia would require 240 hectares of cane to grow, i.e. productivity in terms of sweetness equivalent per hectare is high.

For a better understanding of the crop and its potential, a review study was undertaken by CQU and is included herein (Chapter 2).

In Japan, *c.* 2000 t of stevia extracts are consumed, in the past 95% coming from raw material produced in China. Nowadays, four major producers in China supply the Japanese market. The market in the US is currently in strong competition with artificial sweeteners, but these are loosing ground in the health war stakes. As this extends to include Australia, the consumer demand for natural sweeteners will escalate, and the soft drink industry is aware of this. It is the intent of this project to determine whether under Australian conditions the crop and product will have any comparative advantage. Given the availability of assured irrigation, year-round growing conditions, and access to the sugar industry's research facilities for pilot-scale extraction, it would appear pertinent to research the crop.

A number of groups have recently expressed interest to CQU in growing the crop but cannot readily find information or planting material in Australia. Groups that have expressed interest include the Dawson-Callide Herb Growers Association (CQ), the Northern Rivers Agricultural Development Association (NORADA - NSW), the Rural Innovators Group (Bowen-Burdekin Region, NQ), Queensland Cotton, and Zinnack Nominees of Kangaroo Island (SA), and from the user side, The Right Food Group NSW, Mackay Sugar, Bundaberg Ginger Beer, and the Australasian Soft Drinks Association.

With the increased incidence of diabetes in Australia and abroad and growing concern over the safety of some chemical sweeteners, there is a need for a natural non-calorie sweetener with acceptable taste and health properties.

Advances in plant selection/breeding, especially in China, Korea and Japan, have enabled the sometimes noticed 'liquorice after-taste' sometimes noticed to be eliminated. Research has also made possible simpler, water-only extraction processes (similar to sugar processing) in place of the older solvent extraction technology.

Overseas, steviosides have been successfully marketed in conjunction with sugar as "double strength" sugars. Marketing with sucrose could enable the sugar industry to provide a complete range of sweeteners including low-calorie and no-calorie products. In soft drinks alone artificial sweeteners (imported) currently replace the equivalent of 40,000 tonnes of sugar a year, valued at over $12 million. Other diet products including cordials, juices, some jams and some sweets also contain chemical sweeteners in place of sugar and are potential markets for stevia. The ability to be used in baking opens the potential for new, low or no-sucrose but sweet tasting cakes, biscuits, pastries etc.

A successful stevia industry in Australia could, therefore, provide benefits as follows:

To primary producers, enabling greater diversification opportunity and returns per megalitre of irrigation water
To the rural sector in the form of additional employment if a commercial extraction plant is established
To the commercial users of artificial sweeteners through reduction of liability should links be established between artificial sweeteners and disease incidence, and to the consumer through potentially reduced health risk than if consuming artificial sweeteners
To the economy through import substitution (reduction of current imports of artificial sweeteners).

It is proposed that a business plan be developed once it is shown that there is realisable potential for stevia in Australia. It will involve all those with a commercial interest in the product. Most likely a levy charged at the point of extraction will provide income to the suppliers of germplasm, and to the funders of the research with profits accruing to growers, the extract or (most likely the sugar industry) and financial benefits to the purchasers of the purified steviosides.

From an ecological perspective the outcome of this project will in the medium term increase opportunity for farming systems diversity, and should lead to greater stability in the medium/longer term.

2. Literature Review and References

2.1 Introduction

The leaves of Stevia, a perennial bush native to eastern Paraguay, have been recorded as a sweetener for many years. It has been largely ignored by most of the world until the last ten to fifteen years. However, there is now rapidly expanding interest in Stevia as a natural alternative to artificial sweeteners because it is not only a 'natural' calorie free product but also has other advantages over the currently used chemical artificial sweeteners, such as saccharine, aspartame and cyclamate.

As part of this increased interest a number of reviews have been undertaken in various countries including: Brazil [10, 46], Japan [142], Canada [21, 37], India [29], Georgia [55], Germany [41], Russia [44], Czech Republic [98], Korea [71], Mexico [49], Sweden [56], California [127]. It was also included in a 1992 review of possible new crops for Western Australia [61]. Interest is also being shown by various natural food/herb consumer groups and individuals on the Internet by way of articles, comments and discussion groups (a search for 'Stevia' will locate many www sites).

This current review is from the standpoint of assessing the potential to establish a Stevia industry in Queensland.

Invariably the reviews have highlighted the attractiveness of steviosides as sweeteners and all endorsed the establishment of a Stevia growing industry. These reports emphasize the extreme versatility of this tropical perennial plant. It has been successfully grown as far as 60^{o} N (St. Petersburg) and frequently in latitudes 35^{o} – 45^{o} N as well as within the tropics.

This review only utilises readily accessible international articles and publications for which English titles (or translations into English) and, often limited, abstracts are available. It may therefore give an incomplete representation of the current status of Stevia production and research. It should be remembered that many more investigations and industry reports will have been written in other languages, especially in Japan, China, and Brazil, and may not be accessible in English language literature retrieval databases. Russia has been working on Stevia since its importation in 1936. It would appear that the only scientific work on Stevia undertaken in Australia is some limited agronomic experimentation within the nursery and herb industry and a 1999 student project at the Gatton campus of the University of Queensland [91].

Most of the more than 1,500 scientific research works on Stevia have originated in Japan, especially since 1970. Japan is by far the most advanced country in the use and understanding of Stevia and its application in the food and pharmaceutical industries. Some of the articles and references used in compiling this review have themselves cited a large number of references, many of which are not repeated here, e.g. [21] – 116 references, [46] – 83 references all pre 1977, [116] – 114 references.

2.2 Background

Stevia rebaudiana (Bertoni) was 'rediscovered' by Europeans in Paraguay in 1888 by Dr M.S. Bertoni. He later botanically described and named the plant (in 1905) in honour of Paraguayan chemist Dr Rebaudi. Stevia is one of the 950 genera of the Compositae (Asteraceae). There are now known to be more than 150 *Stevia* species but this is the only one with significant sweetening properties; other species do contain other biochemicals of interest.

It is native to a relatively small area of eastern Paraguay (on the Brazilian border) where its leaves have been used by the local Guarani Indians as a sweetener for many hundreds of years. They especially use it in the local green tea ("Mate" tea – *Ilex* sp.), as well as with otherwise unpalatable medicinal and other drinks. In its native state it is a perennial herb, living 3 – 5 years, with variable appearance up to about 0.7 meters tall.

Stevia rebaudiana has been taken to many countries since first described by Bertoni and has subsequently been grown in latitudes well north of its native Tropic of Capricorn latitude (Table 3). Mechanized agricultural production systems have not yet been developed and early initiatives to develop a sweetener industry based on Stevia usually lapsed as sugar became readily available (e.g. in U.K. during WWII).

Interest has been rekindled in more recent years, especially in the developed world where diet conscious consumers seek a natural low-calorie sweetener as an alternative to chemical sweeteners. Stevia products are used commercially extensively in Japan, using locally grown and imported (mainly from China) dried stevia leaves, where (at over 2,000 tonnes refined product) they make up over 40% of the non-sucrose sweeteners (the others being fructose, syrups, honey, etc) and 5 – 6% of the total sweetener market. Chemical sweeteners such as aspartame are banned in Japan [138]. In most other countries where it is used it is mainly used directly by consumers, rather than commercially. Domestic consumers add dried leaves, liquid extracts, crystals or powders to their drinks and cooking as a 'herbal' supplement.

Some of the institutions around the world that have undertaken research and/or appraisal studies on Stevia have included visits to Paraguay for collection of seed and plant material in its wild, natural environment. [15, 54, 127, 137, 141, 150]

The main stevioside[1] producing countries are China and Paraguay with adjacent parts of Brazil (Table 1). China is the main supplier to Japan, who are the main commercial producers and users of steviosides. Paraguay/Brazil is the main centre for the production and distribution of Stevia products direct to consumers via the health food and herbal product outlets and by direct mail order sales around the world. There are a number of processors in Paraguay and Brazil who have company plantations of 2 – 300 hectares or more as well as numerous small-holder suppliers [45, 116]. Total production from these processors is not known. Recorded uses for Stevia products are as extensive as for sugar, with the main ones now being low-calorie diet drinks and table-top sweetener use for home cooking (Chapter 5).

Table 1

Some Countries Where Stevia is Grown and Researched

Country/Location	Commercial Production 1.	Research	Non-Agric Research.	Approved for use
South America-Paraguay	++	++	++	++
Uruguay / Brazil	++	++	++	++
Central America	+			+
Mexico	+	+	+	+
United States of America		+	++	
Canada		+	+	
China	++	++	++	+++
Vietnam	+	++	++	+
Taiwan	+	++	+	++
Japan	+	++	++	+++
South Korea	+	++	++	++
Thailand	+	+	+	+
Malaysia		+	+	+
Indonesia		+		+
India		+		
Georgia		+	+	+
Russia	+	++	++	+
Ukraine/Moldova	+	+	+	
Spain		+	+	
Italy		+	+	
United Kingdom		+	+	
Germany		+	+	+
Sweden			+	

1 Commercial production excludes small quantities grown for domestic use.

[1] Stevioside/s is commonly used as a collective name for the glycoside sweeteners from the Stevia leaves and Stevioside is also one of the four main glycosides. In this review, when used as the collective, stevioside is spelt with a lower case s, and when referring to the specific glycoside Stevioside is spelt with a capital S.

2.3 Plant Physiology and Chemistry

2.3.1 Chemistry

From the time *Stevia rebaudiana* was first described in the early 1900's the chemistry of the Stevia sweeteners has attracted interest from chemists and later biochemists. In 1908, crystals of $C_{42}H_{72}0_2$ were suggested as ingredients. In the 1930's steviol, the precursor of the sweetener, was identified. In the 1950's and 60's the structure of the main sweet diterpenoid glycosides (steviol glycosides, $C_{38}H_{60}0_{18}$) were established. The chemistry of these compounds is well researched and reviewed. One of the more recent reviews is by Brandle, et al., 1998 [21].

The two main glycosides are Stevioside (St), traditionally 5 – 10% of the dry weight of the leaves, and Rebaudioside A (R-A), being 2 – 4%; these are the sweetest compounds. There are also other related compounds including Rebaudioside C (1 – 2%) and Dulcoside A & C, as well as minor glycosides, including flavonoid glycosides, coumarins, cinnamic acids, phenylpropanoids and some essential oils [44, 75, 80, 81, 97, 121, 122, 124, 144, 156]. New minor compounds continue to be identified [73, 82].

The structure and biosynthetic pathways for these compounds have been studied at length [21, 42, 66, 77, 80, 81, 118, 128]. C nuclear magnetic resonance (C-NMR) spectrometry has been used to determine the chemical structure of the glycosides [148]. The biosynthesis is linked to the biosynthesis of gibberellin growth regulators; Stevia thus may be a potential source of these compounds. There can also be significant quantities of gibberellins in Stevia [7, 8, 33]. Biosynthesis of the sweet compounds takes place in green tissue (chloroplasts) and so they are in the leaves and green tips of the plants. As stems mature and lose colour any steviosides present dissipate. These Stevia glycosides are stable at high temperature, to 200 ^{o}C, and in acids. The essential oils are generally volatile in boiling water [97], are concentrated in glandules in plant tissue (including stems) and could possibly be extracted separately [124].

There are suggestions that the stems and flowers could contain some of the compounds which may give Stevia some of its other properties, especially flavour enhancers (flavanoids), odour enhancers and organoleptic substances [40, 116, 122]. Enzymatic modification (breakdown) of stevioside to produce a flavonoid – rubusoside – is possible [66]. The structure, development and chemical content of Stevia roots have also received attention, often associated with culturing procedures [166, 167, 172].

2.3.2 Relative Sweetness

The relative sweetness of the two main glycosides, Stevioside (St) and Rebaudioside A (R-A) are well established. Stevioside traditionally makes up the majority of the sweetener (60 – 70% of the total) and is assessed as being 110 – 270 times sweeter than sugar. It is also responsible for the after-taste sometimes reported (licorice taste). Rebaudioside A is usually present as 30 – 40% of total sweetener and has the sweetest taste, assessed as 180 – 400 times sweeter than sugar with no after-taste. The ratio R-A/St is the accepted measure of sweetness quality – the more R-A the better. If R-A is present in equal quantities to St (or more), it appears that the after-taste is eliminated. The minor glycosides are considered to be less sweet, 30 – 80 times sweeter than sugar. [11, 19, 39, 69, 116, 158]

The exact mechanism of the sweet taste is not known. Chemicals with only slightly different structures are often not at all sweet and sometimes quite bitter [77]. The sweetening effect of these compounds is purely by taste; they are undigested and no part of the chemical is absorbed by the body. They are therefore of no nutritional value [64].

One teaspoonful of dried leaves (finely ground) are claimed to have a sweetening value equal to one cup of sugar. In domestic use the sweetening powers claimed, for suggested 'recipes' and for some table-top products, are inconsistent and often extreme.

Stevia products can be used in conjunction with other sweeteners, in which case the sweetening value is compounded and not diminished. When added to sugar it can give 'double strength' sugar and is sold in this form in some countries [49, 79]. In Japan and China it is frequently mixed with other sweeteners.

2.4 Leaf Analysis and Assay Methods

Along with the interest in the biochemistry of the sweeteners there has been parallel interest in methods for the analysis of the glycosides for both determining what sweeteners are present and in what concentrations. Determining the level of the various sweeteners is an important part of the breeding and selection process. Rapid assay methods are required to screen crossbred lines, to assess responses to variable management treatments and to determine optimum time of harvest etc. The very close correlation between stevioside content and soluble carbohydrate content could allow for simple estimation of stevioside content in dried leaves [113].

A range of analytical techniques have been used to assay Stevia leaves; these include various types of chromatography, electrophoresis, magnetic resonance spectrometry, spectroscopy and enzymatic determination [99, 107, 148, 170]. Extracting the sweeteners from green or dried leaves can be readily done using boiling water with extraction efficiencies up to 98% achievable [6, 113]. In commercial extraction and some analytical techniques methanol and water is sometimes used. Recent commercial extraction procedures do not require methanol [138].

Chromatography is the main assay technique used and many refinements to procedures have been developed to improve accuracy and efficiency. Procedures used include thin layer chromatography [103, 120] (which registered low stevioside %), droplet counter current chromatography [76] and, more popularly, high performance liquid chromatography (HPLC) eg. [170]. These procedures have been reviewed in some detail [4, 21, 36, 46].

Analytical procedures, including HPLC, can assay stevioside levels in plant and food samples down to concentrations under 10 mu g/ml (10 ppm) [103] and absolute quantities as small as 0.6mu g. [6]. HPLC is also useful to analyse and quantify possible stevioside metabolites [6, 65]. HPLC has proved to be a reliable method for the separation and quantification of the different glycosides [3]. A range of adsorbents have been used in chromatography determinations including silica gel and amino bonded columns (most common) [2, 3, 17, 21, 32, 36, 59, 65, 78, 96, 111, 120, 126, 155]. HPLC systems developed are capable of analysing large numbers of samples quickly [18]. Near infrared reflectance spectroscopy (NIRS) has also been used successfully for stevioside determination in ground dried leaves [111, 112]. NIRS determination has the potential to enable non-destructive screening of large numbers of samples very quickly.

2.5 Uses

The established uses for Stevia products cover all those of artificial low-calorie (non-sucrose) sweeteners, aspartame etc.[2], as well as most other purposes for which sugar can be used. The primary use is as a sweetener to enhance the palatability of foods and drinks. Unlike aspartame, Stevia sweeteners are heat stable to 200 ^{o} C, are acid stable and do not ferment, making them suitable for use in a wide range of products including baked/cooked foods. In some food uses its lack of bulk make it unsuitable to replace all of the sugar in recipes, such as confectioneries, icings etc. In addition to sweetening foods stevia extracts can increase the palatability and attractiveness (enjoyment) of food through enhancement of flavours and odours [66].

Stevia products also have beneficial uses as herbal and medicinal products and for some more unusual uses, e.g. in tobacco products. A fermented extract of stevia showed bactericidal activity against food borne bacteria including *E. coli* [153]. Some of the accepted uses are given in Table 2 (references for these are not listed here).

[2] Artificial low-calorie sweeteners used in Australia include aspartame (nutrasweet), saccharin, cyclamates, acesulphame K, sucralose, alitame and thaumantim (a plant extract).

Table 2

Some Uses for Stevia Products and Extracts

Food and Culinary Uses

Table top sweetener – for tea, coffee etc
Soft drinks, cordials, fruit juices
Ice-creams, yoghurts, sherbets
Cakes, biscuits
Pastries, pies, baking
Jams, sauces, pickles
Jellies, desserts
Chewing gum
Candies, confectioneries
Sea-foods, vegetables
Weight-watcher diets
Diabetic diets
Flavour, colour and odour enhancers
A source of antioxidants
Alcoholic beverage enhancer (aging agent and catalyst)

Medicinal Uses

Toothpaste, mouthwashes – plaque retardant/caries preventor
Skin care – eczema and acne control, rapid healing agent
Diabetic foods and weight loss programs
Hypertension treatment and blood pressure control
Calcium antagonist
Bactericidal agent
Pill and capsule additive to improve taste

Other Uses

Tobacco additive and flavourant
Production of plant growth regulators (potential use)

2.6 Agronomy

2.6.1 Environment

The natural habitat of Stevia is semi-humid subtropical on the Tropic of Capricorn (22 – 23^{o} S latitude), 200 – 400 meters above sea level, with 1,500 – 1,800 mm of rain and temperature extremes of minus 6^{o} C to plus 43^{o} C. It naturally grows in low lying areas on poor sandy acidic soils adjacent to swamps, and so is adapted to and requires constantly wet feet or shallow water tables. [37, 116, 127] Under cultivation and on more fertile soils, the mature plant can be larger, up to 1.8m with up to 20 branches per plant [70].

Vegetative growth is reduced when temperatures are below 20 degrees and when day length is less than 12 hours. Increasing day length to 16 hours and increasing light intensity can increase vegetative growth and stevioside levels [104, 169]. Flowering is photoperiod dependent and is enhanced by reducing day length and short day length. Responses of flowering, and also stevioside content, to day length appear to be variable with

some cultivars/selections being obligate short-day plants [21] however some lines appear to be photoperiod insensitive. Early flowering lines tend to have higher stevioside content but lower total yield [142].

Stevia has been successfully taken to a wide range of climatic locations around the world (Table 3) and apparently grown successfully, although often by using vegetative propagation methods and seedling establishment in a greenhouse before planting in the field.

Shading has been shown to reduce growth [135]; yet in China highest yields were recorded at mid-latitudes (32 degrees north) in an area with generally limited sunshine hours (heavy cloud cover) for much of the growing (wet) season [129]. Therefore it is likely that factors other than latitude and solar radiation usually limit production. Humidity is reported to be important at some locations as the plant has poor tolerance to water stress (it often visibly wilts under hot dry conditions even with good soil moisture levels). Rainfall in many locations is supplemented by irrigation to avoid any moisture stress. Only in the more humid wet areas may rainfall alone be sufficient for adequate growth for commercial production. Climatic requirements, of day length and temperature, are different for maximum vegetative production and for maximum flowering and seed production [61].

Soils of the natural habitat are generally low fertility, acidic sands (pH 4 – 5) with shallow water tables and little organic matter, where plants grow to 0.6 – 0.7 meters in height. Under cultivation on better, more fertile soils, growth can increase to 1.0 meters and even to 1.8 meters [70]. Soils should be well drained but with reasonable water holding capacity and preferably with pH 5 – 7; alkaline soils should be avoided [116].

2.6.2 Varieties, Breeding and Selection

In the wild populations of *Stevia rebaudiana* there is great variation in phenotype and in leaf analysis. The collections made as part of the various breeding and selection research programs have invariably included a range of genotypes and selections of plants with differing levels of steviosides in their leaves e.g. Shock planted out 200 lines for survival testing and screened 17 lines for productivity [127]. The stevioside content of leaves can vary substantially (4%-16%) between individual plants even after a selection program has been operating for some time [15, 109].

Table 3

Climatic Features of Some Stevia Agronomic Research Locations

Ref	Location	Latitude Degrees	Rainfall -mm	Altitude -meters	Topo-graphy	Comments
[44]	St Petersburg Russia,-Voronegh	60 N 52 N		<200 <200	Plains Plains	
[55] [129]	Moldova/Ukraine North China	47 N 45 N	600	< 200	Plains	 Hielongjiang Prov.
[37] [55]	Canada Georgia	43 N 43 N		250-300 < 200		irrigated Black Sea Coast
[127] [88]	California Korea – Suweon	38 N 37.5 N	 1,250	<200 <100		Davis, irrigated
[129] [98] [49]	Central China Japan-Kyushu isl. -Okanawa Mexico	32 N 32 N 26 N 25 N	2,000 2,475 2,150 200	< 200 <50 <50 < 200	Coastal Coastal Island Coastal	Jiangsu Prov. tropical conditions irrigated
[133] [98] [29]	Taiwan Thailand India – Bangalore	24 N 18 N 13 N	 1,260	 300 930	 Uplands Uplands	 6mth dry season
[53] [98]	Indonesia – Bogor - Surakata	7 S 7 S	1,800 2,300	400 1,000	Slopes	 3mth dry season
[98] [116]	Brazil – Maringa Paraguay –native locality	23 S 23 S	1,620 1,500 -- 1,800	500 200-400	Uplands Inland - valleys	400km from coast Amambay Mountain Ranges

This natural variability could be partially due to the largely self-incompatible nature of the flowers. The variability goes as far as some variability in chromosome number [58]. The heritability for yield, leaf/stem ratio and stevioside content, have been found to be high at 75% - 83% [19, 20]. These high heritabilities enable selection and breeding programs, aimed at higher yield, to achieve substantial gains. Some interesting correlations have been found which assist selection programs. Plant leaf yield is proportional to branch number, leaf number and (not always) plant height [22, 23, 131]. Total stevioside content is positively correlated with leaf/stem ratio [150]. Leaf thickness is positively correlated with R-A/St ratio [133]. The total stevioside content in leaves at the seedling stage and when mature are not correlated, making plant selection at the seedling stage ineffective. High R/A content is also linked to large leaf area, high net photosynthetic rate, high chlorophyll and protein content [161].

Countries that have been researching Stevia for some time, especially Japan, China, Korea, Taiwan and Russia, have all reported success in their breeding/selection programs and have at various times released new varieties with improved stevioside content and higher yields (Table 4). Most breeding programs are based on cross breeding and selection. Irradiation with ^{60}Co gamma rays has been to induce variation in breeding lines [154]. Vegetative propagation and cloning has frequently been used to multiply individually selected plants. Some of these selections, although very high yielding, are self-incompatible and can only be reproduced vegetatively [87]. This limits their commercial use, although they may be useful for breeding new hybrids.

Table 4

Some New Variety/Cultivar Selections & Releases

Year	Location	Ref	New Variety	Features
1979	Korea	[89]	Suweon 2	Yield +22%, Steviosides + 12%
1982	Korea	[87]	Suweon 11	Thick leaves, high R-A%
1989	China	[129]	Yunri, Yunbing	
1995	China	[130]	Zongping 1	Highest R-A % and St%
1994	Taiwan	[133]	K1, K2, K3.	High yield, better R-A/St ratio
1994	Indonesia	[141]	BPP 72	
1996	China	[161]	SM4	Yield up 1.5%, R-A/St ratio up
1996	Russia	[84]	Ramonskaya Slastena	

Even with clonal reproduction there can be some variability – an Indonesian study [115] showed variations in DNA fingerprints between six groups of plantlets from an invitro culture indicating some clonal variation. In China in a sample of plants from one clone, St% varied from 1.48 – 6.98 and R-A% from 4.5 – 12.1, with total stevioside varying from 10.26% - 19.57% [63]. With the high heritabilities recorded, and the ability to select for high yield and high stevioside content in the leaves at the same time, plus the high level of natural variability due to constant out-crossing, it is not surprising that breeders have been able to double the level of sweeteners in leaves (from 3 – 9% in the wild population to 15 – 20%in some lines) and alter the R-A/St ratio from 1:2 to 2:1 [63, 129] (table 5).

Table 5

Some Stevia Leaf Analyses

Ref	Location Cultivar	St %	R-A %	R-A/St Ratio	Total Sweetener
[116]	Paraguay – average	8-14	2-4	0.4	10-15%
	Paraguay – typical	5-10	2-4	0.4	9-15%
[150]	Paraguay – wild				10.2-13.5%
[63]	China – average	6.44	3.86	0.6	10.3%
	-- select'n 508	5.73	12.03	2.1	17.9%
	– J2/8	3.78	12.15	3.2	18.3%
	- J2/23	6.98	10.47	1.5	19.57%

2.6.3 Propagation

In the wild, Stevia regenerates from seed, from the rooting of plant stems touching the ground (and trampled into the ground) and from regeneration at the base of the plant (crown division). Seed germination is notably very poor, commonly due to infertile seed. Some plant varieties/selections produce virtually no viable seed due to their self incompatibility.

Under cultivation Stevia can be propagated by seed, by tissue culture and by vegetative cuttings (and plant separation). A comparison of these three method [147], showed no significant difference in growth nor glycoside content between the three methods; cuttings showed least and seed greatest variation between individual plants in chemical composition.

Seed Propagation. Using seed to establish crops of Stevia is more successful in tropical climates where there is no climatic restriction on the length of the growing season. In northern climates the shorter growing season necessitates seedling establishment in a glass house/green house prior to the growing season. Germination and establishment from seed is often poor and sometimes unsuccessful [123]. Stevia flowers need to be fertilised by pollen from another plant to produce viable seed. A high density of bees (3-4 hives per hectare) is recommended for good seed production [116]. Harvesting of immature seed may also contribute to poor germination [37]. In Australia the limited range of plant clones show poor seed fertility and germination such that nurseries claim that plants can't be grown from seed [91]. Only vegetative propagation is used to supply the demand for plant specimens.

Where seed production and fertility has been studied, research reports suggest that for selected lines high germination rates are possible e.g. [26]. Timing of flowering and seed harvest and pollination methods play an important role [102, 138]; rain at flowering can also reduce seed setting. Shade can reduce total growth, delay flowering and reduce the rate of flowering [135].

Fertile seeds are usually dark coloured whereas infertile seeds are usually pale [46, 116]. Seeds are very small (1,000 seeds weigh 0.3 – 1.0g) and as a result seedlings are slow to develop, reaching a size suitable for transplanting to the field at 45 – 60 days [21, 37, 116]. Seeds are usually germinated in a greenhouse where a range of bedding materials have been trialed [25].

Direct seeding to the field is not practiced but may be a requirement for large-scale commercial production. In one trial [86] plants from seeds were less productive in the first year than those from cuttings.

Germination rates vary greatly. It can take 4 – 6 days to reach two thirds of the final germination of 62 – 90% at 25 ^{o}C [26, 34, 127, 145]. Germination requires at least 20^{o}C and often more than 25 degrees; light generally increases germination. Accelerated ageing (40 degrees on moist paper) for less than 24 hours can accelerate germination but reduce total germination [148]. In Japan seed viability remained for up to 3 years if stored under low humidity and in darkness [72]. This contrasts with claims of optimum storage at 0 degrees still

producing a 50% loss of viability after 3 years [21]. In one experiment field-collected seeds resulted in 34% germination compared with 90% for greenhouse-collected seeds [24].

Total quantities of viable seed produced in one year are uncertain. One reference to seed yield suggests up to 8 kg/hectare, which at 50% germination would be sufficient for 250 hectares of crop [91]. Another reference suggests that in North China a seed yield of 67 kg/ha (9 jin/mu) is possible in conjunction with a dry leaf yield of 1387kg/ha (185 jin/mu) [70].

Vegetative Propagation. Considerable research effort has been applied to vegetative propagation including tissue culture/invitro multiplication. Cuttings of new stems and shoots can be propagated successfully [55, 86, 112, 127] and rooting of cuttings can be stimulated, but not always, by the use of growth regulators [16, 27, 83, 85, 173]; some growth regulators can sometimes influence (increase) the concentration of steviosides in the leaves [1, 35]. The size (number of leaves) of cuttings and day length during cutting can influence rooting and growth [174]. Cuttings taken in late winter (August in Brazil) rooted better than those taken at other times [27]. The location on the plant from which cuttings are taken can also affect growth and rooting, cuttings from the top half and with four internodes performing best. [152]

For tissue cultured propagation many different parts of the plant can be used successfully – leaves, auxilliary shoots, root-neck sprouts, shoot primordia, internodal explants etc [5, 13, 14, 38, 47, 57, 85, 105, 143, 168]. Invitro multiplication has been used frequently to multiply individually selected or bred clones and successful procedures have been documented [16, 25, 47, 48, 57, 85, 110]. Variations to standard micropropagation media have been studied to maximize Stevia plant survival rates [13]. A range of multiplication and rooting media have been evaluated [38, 85]. Techniques and equipment for large-scale mass propagation have been developed, especially in Japan [5].

2.6.4 Cultural Practices

Fertiliser. Fertiliser requirements are moderate for Stevia, partially due to its adaptation to poor quality soils. Fertiliser trials show yield reductions at high rates of fertiliser [52, 83, 86, 88]. Even though theoretical nutrient usage of Stevia might be 105 kg N, 23 kg P, 180 kg K per hectare based upon nutrient removal through harvesting, maximum rates of NPK 40-20-30 for India are suggested [28]. Higher rates with split dressings, particularly for nitrogen, are also suggested [37, 116].

There appears to be no unusual requirements for minor elements [94] and nutrient deficiency symptoms are typical of other crops [93, 125, 159, 160, 171]. There can be variable interaction between some nutrients at different availabilities [92]. Fertiliser requirements for pot trials have also been established and are not high [9, 52].

Plant Densities. Most cultural systems involve transplanted seedlings and so densities less than the optimum are usually recommended to save costs. Densities of 80 –100,000 plants per hectare on row spacings of 45 – 65 cm are generally recommended [37, 43, 116] with densities up to 160,000 suggested for higher yields [108]; in one trial in Georgia, 60,000 gave highest yields [55]. With multiple harvest systems plants develop new tillers from their crowns and so ratoon crops are likely to be more dense and the additional cost of planting higher densities may not be economic. In their first year crops can easily lodge and stems break; yet ratoon crops have stronger stems and do not suffer from lodging [142].

Irrigation. Supplementary irrigation is generally assumed to be essential to avoid any water stress on plants unless the growing area has reliable rainfall throughout most of the year. Spray irrigation has been suggested [37] but this could encourage leaf diseases and reduce seed set (where seed is required). Frequent irrigation is required to maintain soil moisture above plant wilting-point, which is suggested to be as high as 80% of field capacity [116]. ‘Any’ moisture stress (not defined) can reduce leaf production [51]. Tolerance of excess moisture could be the reason why cultivation on flat sites gives higher yields than on slopes [53, 60]. Stevia could be suited to the use of underground trickle irrigation systems (‘Tee’ tape)

2.6.5 Pests

Weeds. Being slow to establish, seedlings are very susceptible to weed competition until well established. Weed control by cultivation before and after transplanting is most commonly used; this is not difficult where crops are grown in low labour cost countries. Herbicides will be important for mechanized production and it

appears that trifluralin is tolerated and so its use may be possible [21]. Black plastic mulch and very high density planting (up to 200,000/ha) have also been shown to be effective for weed control [12].

Insects. Insects do not appear to be a problem. Stevia has shown clear aphid resistance – the sweet taste being a possible deterrent to insects [104]. Slugs have reportedly attacked new tillers after winter dormancy [127].

Diseases. Diseases do not appear to be a major problem either, although there are reports claiming to record the first known incidence of Sclerotinia, leaf-spot Septoria and black-spot Alternaria infections [31, 67, 68, 95]. In Russia diseases observed over a 50 year period included Alternaria, Botrytis, Fusarium and Rhizoctonia [175]. Glasshouse specimens at Gatton, Queensland carried unidentified fungal diseases. In Paraguay potential diseases are identified as *Alternaria steviae* (large black spots), *Septoria steviae* (small dark spots), *Rhizoctonia solani* (stem spots and wilting of leaves) and *Sclerotinia rolfsii* ('white silk' round the plant stem). Spraying for control is sometimes undertaken [116].

2.7 Harvesting

2.7.1 Procedures

In colder climates (with severe winters) only one harvest per planting is possible. Under these circumstances the harvest is when yield is greatest and can be at the onset of cold weather [37]; early harvesting will reduce total yield [132]. By covering the harvested crowns of the plants (with polythene etc.) plants can survive over winter and production for a second season can be possible [55]. Harvesting should be before flowering at flower bud appearance as the stevioside content of the leaves falls when flowering commences [15, 61]. Under late autumn conditions artificial drying is generally required to reduce plant moisture content to less than 10%. Harvesting is effected by cutting the whole plant 5-10 cm above the ground [43]. The time of the onset of flowering varies with variety, with some varieties flowering once day lengths decrease.

Crushing of the main stem (conditioning) at harvest does not appear to be a normal practice, even though this would reduce drying time.

In warmer climates where some plant growth is possible for most of the year (and plants do not die over winter) more than one harvest per year is normal. In Paraguay/Brazil three harvests a year are normal [43], often with a cleaning harvest after winter used for vegetative propagation purposes. In mid-summer harvest intervals can be less than two months [116]. In India four or five harvests a year are possible and in Indonesia up to seven harvests have been possible per year [12, 29].

2.7.2 Crop Yields

Commercial yield figures are not reported other than in general terms, e.g. 1,600 – 2,000 kg dried leaves/ha/year. Experimental yields suggest that systems with multiple harvests a year will give higher yields and, where ratoon crops are grown, harvests in the second and third years are likely to be greater than for crops requiring replanting each year [106]. A range of yields achieved under experimental conditions is given in Table 6. Commercial large-scale growers should expect only 50 – 70% of experimental yields. Yields for second year (ratoon) crops can be higher than for first year crops [106].

Table 6

Trial Crop Yields

Trial Location	Ref	Yield Total	T/ha-dryleaf	Steviosides			Kg/ha stevios's
				Total	R-A%	St%	
California	[127]	9.0	3.6	7%			252
Russia	[83]	13.8	5.5	*9%			495
Russia	[44]		1.4-3.0	*9%			125-270
China - V9	[129]		1.40	20.4%			265
- average				12.4%			186
- Jiangsu							267
North China	[70]		1.3 plus 67kg seed				
Indonesia	[141]						
India	[29]		4@2.0	est. 9%			720
Canada	[37]		2.85	15-20			425-570

* - not given; assumed value used to calculate kg/ha steviosides

2.7.3 Post Harvest

The usual procedure is to harvest the whole crop green and transport it to drying facilities: sun drying or (artificial) drying kilns. With low humidity, sun drying of a thin layer of cut plants can be quite rapid (9 – 10 hours) to reduce plant moisture from approximately 80% to 10% [116]. Kiln drying can take two days [37]. Fast drying is likely to give 'better quality' dried leaves. If cut plant material is not dried quickly leaf quality can deteriorate by oxidation, losing up to one third of stevioside content after three days [116]. High temperature during artificial drying can also lead to loss of content. A green dried leaf colour is desirable and represents good quality (as for hay production).

After drying, leaves are stripped from the stems by hand or a mechanical thrasher/separator before leaves are packed for transport to a processing facility. Stems of Stevia plants contain little or no sweeteners, although it is suggested that they may contain some flavour enhancers, odourisers and other agents of potential use for improving food-stuffs or alcoholic beverages [116].

2.8 Processing and Manufacturing

The traditional method of use by the Paraguayan Guarani Indians was to dry the leaves and to use them to sweeten teas and medicines or to chew the leaves as a 'sweet treat'. Stevia was regularly used in drinks many times a day, not just occasionally, with no side effects.

The use of dried leaves (pieces or powdered) is not unacceptable in domestic cooking but does leave a sediment in clear drinks etc and can also leave a green colour. There can also be an unpleasant aroma associated with the dried leaves. Appropriate processing of the dry herbage can remove this aroma which is due to specific leaf compounds (not steviosides) [157]. Aqueous extracts of the leaves – boiled in water, cooled, then strained (filtered) – are preferred in many situations and are better suited for controlled levels of sweetening.

Crystalline powders and extracts are preferred in commercial situations as they have a fixed known sweetening value. Fixed concentration liquids are also acceptable. There are a number of patented refining processes registered in Japan. They generally use the four basic steps:

(i) dissolving the sweetener in boiling water or other solvent
(ii) ion-exchange separation
(iii) filtration with precipitation/coagulation
(iv) crystallisation and drying.

Methanol appears to be used in most extraction and purification processes in Japan, presumably to improve extraction efficiency and facilitate the separation of individual steviosides. This use of methanol, even though it is all removed from the final product and does not chemically alter the product, appears to be the reason for the U.S. FDA (Food and Drug Administration) not classifying Stevia extracts as natural and GRAS (Generally Regarded As Safe) food-stuffs, but as food additives. More recent processing methods using water filtration procedures do not use methanol and so produce a more 'natural' product. [21, 138] Newer factories in Brazil use water only extraction procedures and claim 96% purity of product eg. 'Stevita crystals'.

Boiling water extraction can achieve 93 – 98% removal of stevioside [6, 113]. The purification and separation of the various glycosides has been studied at length, especially in Taiwan and Japan but also USA, with resin adsorption and ion-exchange being commonly used [76, 98, 154, 157]. Reverse osmosis and ultrafiltration processes can also be used [50]. Some extraction methods have been designed to maximize R-A percentage [62]. The need to separate the various steviosides could diminish as the ratio of R-A%: St% in the leaves is increased by plant breeders from under 0.8:1 to over 1.2:1.

In pure (crystalline) form the stevioside mix will be 250 – 300 times sweeter than sugar and therefore could be valued at $75 – 90 per kilogram (cost of equivalent sweetening quantity of sugar at $300 per tonne). For sale purposes crystals are often diluted with a bulking agent such as lactose, maltodextrin or even sugar to produce a product with sweetening value of double, four or even more times that of sugar [79].

2.9 Health and Safety

Although Stevia has been used without any problems for many years (hundreds) in its native Paraguay and in other countries for lesser periods, health and safety issues have been receiving considerable attention in the last twenty years. There has been considerable media attention in the U.S.A., including claims and counterclaims before the US FDA. Many of these claims relate to its potential competitive position in relation to aspartame. Stevia products have been approved for use in the U.S.A. as nutrition supplements although many protagonists claim it should be granted GRAS status in the same manner as tea, coffee, sugar and fruit and vegetables etc. The general safety of steviosides could be largely due to the fact that they are not broken down nor absorbed in the digestive tract [64].

In Japan in particular, where artificial chemical sweeteners are not approved, and in other countries, there have been many toxicology safety studies conducted [45]. Among studies carried out are some to confirm the safety of Stevia for diabetic use [119, 151], to show dental benefits in the form of plaque inhibition and cavity reduction [45], to investigate carcinogenicity and mutagenicity (if any) in animal testing [117, 154, 155]. The safety of feeding to animals, chickens and humans has also been confirmed by a wide range of studies [100, 101, 102, 119, 134, 136, 139, 140, 162, 163].

Studies on food safety, including an extensive review of literature, undertaken prior to 1982 [74, 90] concluded that Stevia leaves and extracts are safe; studies since then confirm this. Possible medicinal uses have been investigated often by using stevia extracts as intravenous infusions in rats; possible effects on glucose metabolism, diuresis, organ weights, endocrine function, etc. have been studied in this way [75, 114, 117, 139, 140]. Stevia extract infusions have also shown some anti-androgenic activity in rats [134]. Likely beneficial effects of stevia extracts, as antioxidants and to relieve blood pressure and hypertension, have also been shown [30, 164, 165].

Steviol (a precursor in the biosynthesis of steviosides) can be produced from steviosides experimentally by using specific bacteria but <u>not</u> in situ in the human body. Steviol can exhibit some toxic and mutagenic activity [149].

2.10 Marketing and Economic Issues

Current markets are restricted to Japan, where there is a well established commercial market, and very unstructured diverse health food, natural food and mail order markets. Firm statistics on market size are not readily available. The Japanese market is variously described as 'over 2,000 tonnes of stevioside' (1996) or '40% of the non-sucrose sweetener market'. The health food/mail order market is primarily supplied from processing companies in Brazil and Paraguay often via distribution centres in the USA.

Information on prices is not published but ranges are suggested by giving a relativity to the price of sugar (on a sweetening value), being usually slightly above to 25% above, the sugar value. Prices of chemical sweeteners are apparently similar to the equivalent sweetening quantity of sugar. Soft drink manufacturers can and do sell diet drinks at the same price as sugar sweetened drinks.

The cost of extracting and refining is not available. Some processors in Japan use patented procedures. In Canada (where 2,200 kg leaf/ha equals cost of production of $8,500) a price of Can$3.85/kg is indicated for dried Stevia leaves [37]. This is approximately Au$40,000 per tonne of stevioside (10% leaf content). One tonne of stevioside is equivalent in sweetening value to 275 tonnes of sugar which, at $290 per tonne of sugar, values one tonne of stevioside at $79,750. Therefore it would appear that the price paid for leaves is approximately 50% of the equivalent raw sugar value. And so, these prices would leave the other 50% for the cost of extraction and refining.

Wholesale prices paid for stevioside crystals in Australia by health-food packers and distributors are approximately $100/kg (Wonder Foods Brisbane). Retail mail-order prices for stevia crystals or powders ('pure' 91%-96% dehydrated water extract) are quoted from US$ 145.00/kg in bulk packs and from $280 up to $615/kg in small 15-60 gm packs. (www, six coy. price lists) There is also a range of stevia blend powders and crystals and also liquid extracts/syrups available. These are generally in packs of less than 120 gm (but up to 450 gm) and include tablets and 'tea bags'. The concentrations of these packs are not given and prices vary enormously.

For Stevia growers, break-even dried leaf yields in Canada are suggested to be 2,200 kg/ha to cover a cost of $8,500/ha. If multiple harvests per year can be achieved with plants producing for 2 – 4 years, costs would be significantly reduced, even if a seedling nursery with transplanting is necessary. Direct seeding would reduce costs still further.

Markets for any Australian production could build on established consumption. The herbal/organic/natural food stuffs market in Australia now sells imported Stevia products and initial local production could replace these imports and then enlarge this market. This Australian market is quite small with individual importers each using only 50-70 kg of stevioside per year; this market is growing rapidly.

Use in diet drinks as an alternative to chemical sweeteners is proven to be viable and commercially produced Stevia products could readily compete with and replace imported chemical sweeteners. The Australian soft drink market is approximately 2.2 B litres a year of which over 20% are diet drinks using approximately $12-14 m of artificial sweeteners a year. In addition to soft drinks, there is a growing market for diabetic and low-calorie food stuffs – baking items, jams, cordials, juices etc. With over 20% of the population expected to be affected by diabetes at some time in their lives this is likely to become a substantial market for stevia type products.

There will also be potential for new sweetening products to be sold, such as Stevia blends or double-strength reduced-calorie sugars. The Australian sugar market is in excess of $300 m annually (raw sugar value, not retail) and if diet/low-calorie uses equated to another 10%, this would represent a potential market of $30 m a year for Stevia products.

2.11 Conclusions and Research Needs

2.11.1 Conclusions

Overseas experience shows that stevia is an adapted plant that could be grown in Central Queensland. When priced on a basis of 50% of the value of raw sugar with equivalent sweetening value, the growing of stevia

(dried leaves) has the potential for high gross returns per hectare of $7,500-$15,000. This would appear sufficient to cover the high cost of crop establishment.

Production should be possible in a number of locations provided irrigation water is available. As relatively small areas of Stevia are likely to be viable, it has potential to be an additional crop on existing farms. It could be added to vegetable production, tobacco, cotton, cane, pasture seed, lucerne or even dairy production. Coastal areas, with higher humidity and lower temperature extremes, are likely to be better suited than inland areas.

The potential for successful variety selection to increase stevioside content and the potential to achieve multiple harvests per year for more than one year enhances the long term prospects.

The safety of stevioside for use in foods has been investigated through numerous studies and is well established. The potential to process stevia as a 'natural' product – without any chemical treatment during production - should enhance the acceptance of steviosides as a natural calorie-free sweetener especially suitable for use in diabetic and weight-loss/control drinks and foods.

Marketing in conjunction with sugar, as reduced calorie sugar blends and calorie-free sweeteners, would appear to offer an attractive marketing opportunity.

2.11.2 Research Needs

Critical areas for which additional knowledge from further research is required before a successful stevia industry can be established are:

(i) identification of adequate varieties that can be established from seed,
(ii) development of reliable procedures for crop establishment from seed,
(iii) development of harvesting and drying procedures essential for efficient mechanisation,
(iv) development of efficient seed production and handling procedures,
(v) identification of appropriate processing procedures that will produce a natural product of reliable quality.

After a Stevia industry is established ongoing research will be required to produce improved varieties and address other issues that emerge.

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3. Minutes of the Rockhampton Meeting and Powerpoint Presentations

3.1 Minutes

Present:-

Andrew Rank	CQU
Rowland Lawrence	Tasmanian Institute of Agricultural Research
Gordon Taylor	Sugar Research Institute, Mackay
Max Bourke	RIRDC
Dale Williams	Tomato/Mango producer, Bowen
Tim Sullivan	Callide/Dawson Herb Association
Ryan De Paoli	Aus Chilli, Bundaberg
David Midmore	CQU
Marie Melksham	Diabetes educator, Rockhampton

Apologies:-

Cliff Flemming & Richard Cowdroy-Ling	Bundaberg Brewed Drinks
Peter McLaughlin	NORADA, Murwillumbah
Graeme Bullock and Les Edge	Sugar Research Institute, Mackay
Terry Piva	CQU
Greg Sutherlands	Mackay Region ACC
Jim Kirchner	Canegrowers Mackay
Geoff Milgate	NQEDB
Anne Brown John	The Right Food Group Pty Ltd
Tony Gribble	Australasian Softdrinks Association
Lorraine Zinnach	Kangaroo Island Producer

Presentations

Following an opening by Max Bourke of RIRDC, in which he spoke of the aims and focus of RIRDC and the New Plant Products programme respectively, David Midmore of CQU presented the background and objectives of the small RIRDC project; to establish whether RIRDC investment in research and development on stevia as a natural sweetener was warranted.

This was followed by Rowland Laurence of TIAR who spoke of another RIRDC project, in cooperation with Botanical Resources of Australia, investigating the feasibility and market potential of neutraceutical products from four plant species, including stevia. Initial trials on stevia in that project related to techniques to improve seed germination, and now four varieties, from Brazil, Canada, France and Australia are to be glasshouse and field trialled.

Andrew Rank then summarised the content of his literature review, and spoke extensively of his recent visit to China, illustrated with photographs which are appended. Detail of the visit is recorded in the accompanying Trip Report, as is that of David Midmore who spoke on his recent visit to Japan. Salient points from China were that various institutes are willing to cooperate, at the seed supply (we have subsequently received seed from China), information exchange level, and even commercial level that production is moving northwards as costs for labour rise in the more affluent south of China, and that growers receive a larger proportion than normal (for agricultural products) of the retail price of pure stevioside.

The key findings from Japan were the moves by the stevia industry to gain official recognition (instead of GRAS – generally recognised as safe) for steviosides (a summary of 114 published articles and a book on stevia food safety have been received), the breeding efforts to raise

rebaudioside-A levels in varieties of stevia (the component having less metallic-type taste in extracted steviosides), and process of extraction for steviosides. David Midmore also spoke of the FSANZ (Food Standards Australia New Zealand– formerly ANZFA) position on the use of steviosides as a food additive (at present prohibited), and the possible steps required to reverse that ruling, and on the likely step to be taken to ensure that a viable industry based upon stevia be established in Australia. These presentations are appended.

Discussions

With the likelihood that 1 in 4 Australians will develop diabetes over their lifetime, the dietary health benefits of natural sweeteners (e.g., steviosides) should be promoted, and their development supported by the health industry. It was decided that the CQU team address opportunity for funding of R & D on stevia from the Heart Foundation, Diabetes Australia, the NH???, Federal Health, and the health industry (Health Insurances) amongst others.

Given that two issues require resolution before the stevia industry can get underway, a parallel approach to resolve the issues was agreed upon.

The first is to determine with FSANZ exactly what will be required to ensure acceptance of steviosides as an acceptable "food additive" or a "flavour enhancer" (ie the latter not a sweetener), and to determine whether the requirements will be achievable with foreseeable budgets and support from the major beneficiaries of such an industry. Information provided to date from FSANZ on the extent of trials and data presentation relate to artificial sweeteners, and not natural sweeteners. It may be possible to seek support of consultants or overseas experts to assist in preparing a submission, based upon overseas applications for the acceptance of steviosides as food additives. To this end, *c.*130 articles relating to testing of steviosides has been received from Japan. The second is to develop production and processing practices that result in acceptable financial returns to growers yet result in a product that is competitive in price and quality with any possible future importations.

Both activity streams will in part be funded by RIRDC, with close liaison with industry partners, and experts from the food an health industry partners (dieticians to review evidence on use of stevia to date; watching briefs by Diabetes groups; monitoring of parallel applications lodged inside and outside of Australia for acceptance by FSANZ of "flavour enhancer" status of steviosides and codes for steviosides as "food additives"). After 12 months from commencement (1 July 2002) a review will determine whether the progress has been satisfactory, and that the constraints to production and acceptance of steviosides will be manageable in the near (2-3 year) future.

3.2 Powerpoint presentation, Rockhampton, December 2001

Workshop on a new rural industry
STEVIA
4 December 2001

- Currently not grown commercially in Australia
- Small market for unprocessed form and as crystals.
- Major market in Japan and production in China
- Option for Australian growers if:

  Domestic market assured

  ANZFA will approve use of 'steviosides'

  Costs of production competitive with imports(?)

Small exploratory project funded by CQU and RIRDC

- **Collate available information on production, processing and use of stevia in**

  *** Japan**

  *** China**
- **Present information to interested parties**
- **Allow for discussion of prospects for new industry**
- **Determine interest and follow-up activities**
- **Develop business plan for industry**

Updates on Japanese use of steviosides, sources and current activities

- Meeting with:-
  Dr Tetsya Sumida Consultant on stevia.
  Mr Kanio Kakegawa of Marusen Pharmaceuticals
  Dr Tadashi Katakami of Dainippon Ink and Chemicals
- Reluctance to offer germplasm/information on Japanese trial sites
- Historical context
  - Japanese production (>38ºN replant annually, <38ºS ratoon 3/4 years)
  - Taiwanese/Korea
  - Indonesia
  - Mainland China (originally in S., now more in Mancharia)
- Apparently daylenghth sensitive, need to select DN types for lower latitudes, or harvest x 2, x 3 per growing season
- Canadian Royal Sweet Technology Company as source of DN types.

Steviosides and quality/safety in Japan

Diploid species, so easy to select individuals and propagate vegetatively

Normal stevioside:rebaudioside – A (S:R-A) 1.0:0.4

New:
- 1:2 var. Hotien
- 1:5 var. Seiten
- 1:10 var. Shynten

Apply for PBR with MAFF (Japan)

Claim no knowledge of g x e on S:R ratio

Can use enzymes to raise rebaudioside proportion

GRAS – but now for companies to show no effect

Chemical composition variable, but resolved and CODEX ALIMENTARIUS contribution for early 2002 (CX 711-34 11-15 March 2002, Rotterdam?)

Japanese information

- *c.*10 companies import crude powder from PRC
- Extracted at 50-60º C (x 2 or x 3 extractions)
- Refined in Japan using resins for main components
- Release of components in methanol, 80% removed in first extraction
- Passed along +ve/-ve ion exchange columns to remove colour impurities
- Vacuum pumping to raise concentration 30-40%
- Spray dry to >3 >5%

Suggestions from Japan

Ensure growers have relevant information.

Match production with market (use sugar as biofuel!!).

Compete with PRC ($20/KG 80% purity).

ANZFA official position on steviosides (1)

- Some published reports have raised concerns about safety of stevia.
- Specifically in relation to reproductive effects
- Indigenous contraceptive effects in Paraguayan Indian women
- Planas and Kuc (1968) but no repeated with stevia extracts nor purified steviosides (Nabors and Gelardi, 1991)
- Melis (1999) used aqueous extract of stevia and induced fertility decrease in male rats, but no effect with pure stevioside

ANZFA official position on steviosides (2)

- Joint (FAO/WHO) Expert Technical Committee on Food additives (1999) unable to recommend acceptable daily intake (ADI) because
- Lack of human metabolics on studies on steviosides and steviol
- Not sufficient testing of mutagenicity of steviol
- ANZFA has requested more studies on these two aspects.

ANZFA official position on steviosides (3)

- Current position

– stevia leaf (whole, crushed) is considered a food and legally sold in Australia, NZ

- Stevioside (or concentrated extract) is not accepted for inclusion in Australia/NZ Food standards code, nor for use/sale in Australia/NZ

Updates on ANZFA ruling on steviosides

- Reasoned debate over interpretation of toxicological profile
- Resource limitations and economic factors should not be allowed to compromise safety
- All reports to be in English
- Acute oral toxicity – comparison with other known toxic agents
- Sub-chromic toxicity of at least 90 days
- Oncogenicity of the compound or any of its metabolities

- Reproductive and developmental studies (in vitro not accepted)
- Genotoxicity test for genetic damage (mutations)
- Plus – toxicity of metabolities, degradation products and impurities?
  * Intolerance to food additives?
  * Alteration of nutritional intake?
  * Toxicokinetics and metabolism, trace product and degradation products
  * NOEL highest dose level with no toxic effect in must sensitive test species?
  * ADI over lifetime “without appreciable risk” 1/100 of that of most sensitive species.

What is required for an Australian Industry

- Security that stevioside can be used as ‘food additive’ or as ‘flavour enhancer’
- Access to high quality stevia varieties
- Assured supply of raw and processed product
- Production practices according to location
- Coordination along the supply/demand chain
  * Centralised processing and marketing
  * Production matching demand

MAJOR ACTIONS

- Parallel approach to:
- 1) Ensure progress in recognition of steviosides as food additives or flavour enhancers
- 2) Develop cost-effective production and processing practices
- Both to be shown to have progressed successfully by July 2003
- Engage industry at all possible levels

Actions (1)

- Verify requirements by ANZFA for recognition of steviosides as food additives
- Engage all interested parties to support above action, in their most effective manner
- Verify whether use of stevioside as flavour enhancer is acceptable
- If necessary, acquire funds for trials on food safety of steviosides
- Develop watching briefs for potential users of steviosides

Actions (2)

- Importation of lines for trialing
- Growing out of imported lines for initial growth assessment, and distribution to interested parties
- Establishment of methodology for qualitative assessment of steviosides with HPLC/NIRS
- Propagation procedures to be established, for larger scale trials (seed and vegetative propagation)
- Production practices: water management/drying
  periodic harvests and quality
  mechanisation
- Processing/extraction: diffusion technology a la
  sugar

3.3 Powerpoint photos from China

1 Stevia stubble, Sep ’01 showing plastic mulch, Zhejiang Province.	2 One & two year old stevia plants – regrowth after second harvest, Nantong.
3 Three year old stevia, harvested & ready for winter mulching, Nantong.	4 Stevia plants in peak growing season, Nantong (Midmore).
5 As photo 3 area, in peak growing season, Nantong (Midmore).	6 Stevia cuttings freshly planted, Nantong.
7 New breeding lines of stevia seedlings, Zhongshan Botanic Institute.	8 Breeding lines grown for seed viability trials, Zhongshan Botanic Institute.
9 Selected stevia plants kept for seed after leaf harvest, Jining, Shandong.	10 Stevia seed crop, Zibo Agricultural Research Institute.
11 Photo 10 crop of seed – some leaves not harvested, Zibo.	12 Specialist’s seed crop approaching harvest, Zibo.

4. Other Relevant Information

4.2 Trip report to China

VISIT TO STUDY *STEVIA REBAUDIANA*, CHINA, 14 SEPTEMBER – 2 OCTOBER 2001

Arrived at Shanghai airport, Sat 15th Sept pm, and travelled directly to Hangzhou by bus, arriving at 7.30 pm.

Sunday 16th – contacted Professor Zhujun Zhu of Zhejiang University Horticultural Department, who promptly invited us to lunch and took us to see some of the sights of Hangzhou. He had previously arranged for a visit to see stevia growing east of Hangzhou.

Monday 17th – by early taxi, we met Professor Zhu at the bus station and then bused to Hushan (Cixi County) approximately 145 km to the east.

We met staff at the Municipal Agricultural Extension Department and discussed stevia production in the area – Zhu interpreting. Stevia was first grown in this area in 1989 and reached a peak in '91-'95. Production has declined since, due to falling prices making stevia less profitable than vegetables. They often replant each year so as to grow a winter vegetable crop when stevia is not actively growing, even though plants will last 3 or 4 years and yields in the second year can be 50% above the first year's. Varieties grown are a mixture – 'normal' or 'common' varieties of approximately 10% sugar content and improved new varieties of 15%. Varieties are a problem, with quality variable when planted from seed, so cuttings are usually used.

Cuttings or seedlings are planted out in April for a first harvest in August and maybe a second harvest before November, although some varieties stop growing and produce seed heads in September/October. Better quality varieties only produce 100 kg of leaf (dry) per mu (one fifteenth of a hectare), while common varieties may produce 150 – 200 kg. Farmers don't get a premium for higher stevioside content in the leaves. Unless new varieties, more suitable to the area (less photo-period sensitive), are found production will continue to decline. No factory operates in the area; leaves usually go to Jining? via agents. Agents also arrange for the supply of cuttings to farmers. The department sends some leaf samples to Nanjing for analysis.

After lunch we visited a nearby growing area; plants were very weedy with not much re-growth since the August harvest. In some plots cuttings had been planted into plastic mulch cover.

Returned to Hangzhou by bus.

Tuesday 18th - Professor Yu phoned early and apologized for not being able to meet, as he was heavily involved in a major four day conference.
Visited Professor Zhu at the University and discussed his general research activities and also some research papers on stevia. Zhu supervises five post-graduate projects including:

- heavy metal accumulation in vegetables (Ni, Cu & Co) and tolerant vegetables with low uptake; there is potential for good funding if results are encouraging
- N uptake and accumulation in vegetables
- photosynthesis rate and stress in tomatoes
- salt tolerance and management – especially in covered 'greenhouse' areas (basic investigations involving plasma ion movement).

Vegetables are a 'National Key Subject' and therefore well funded – last year he received one million Yuan for equipment purchases.

Zhu has some capacity to be involved in stevia if a suitable project emerges; the University has purchased access to major literature data bases (Chinese and others), so he has cheap access to electronic articles if required. Had a short tour of part of the campus. It is very attractive, 240 hectares in the middle of the city area and includes paddy fields, green houses and vegetable plots, large mulberry plantations and ornamental gardens, lakes etc.

Tuesday evening boarded a boat for overnight (230km) to Wuxi via the Grand Canal and Tai Hu (lake).

Wednesday 19th – arrived at Wuxi early am and caught a bus to Nantong 110km, via ferry over Yangtze River. At Nantong, met with Dr Xu Gang and a colleague, Dr Yu, who travelled out from Nanjing. Xu Gang spent one year at Rockhampton and is now at the Institute of Vegetable Crops, Jiangsu Academy of Agricultural Sciences. Dr Yu had worked at Nantong Farm previously and knew its location, staff etc. The desire to visit Nantong Farm arose from two papers reporting trials there. In one (a 1989 paper), Nantong had the highest stevia leaf yields for most varieties out of nine locations around China. The other paper (1995) described variety selections at Nantong Farm which had R-A/St ratios over 2:1 and total stevioside up to 17%.

Thursday 20th – drove out to Nantong Farm, approximately 40 km. A large production, research and demonstration farm of 50,000 mu (3,330 ha) owned by the Nantong Municipality. It appears that there is virtually no research being done there now; the researchers have all retired. Stevia has been a major crop in the past, up to 2,000 mu, but there is now only 3-400 mu (20-26 ha), reduced due to lower demand and price from Japan. Stevia grown on this farm is sent to Shanghai and then Japan; there is some processing in Shanghai at a Japan/China joint venture factory. Only a few private farmers grow stevia near the farm using cuttings from the Farm and they must sell their leaves via the Farm to Japan. Only Japanese supplied varieties are grown, all vegetatively propagated, a new variety every three or four years. They say the price for leaves is now very low, 12-14 Yuan/kg(?). They don't do any leaf analysis locally and don't know what stevioside content the crops have. Cuttings are planted and last three or four seasons, with two harvests per year, June-July and September-October (the second was finished this year well before 20th September). The plants are usually heavily mulched with straw and then covered with plastic for the winter and uncovered in March (seems a bit excessive for a coastal area 32°N latitude). Yields in the first year are approximately 100 kg/mu (1,500 kg/ha) and 200 kg/mu in the second and third year (they suggested that the Government controlled production levels and prices!). On inspection of the field area saw first, second and third year crops in the process of being cleaned up for winter mulching. The land is very productive and receives high rainfall; drainage is usually a problem but it was unseasonably dry at inspection. Rice yields in the area are 500-700 kg/mu (7.5 – 10.5 t/ha). Annual nurseries were being established by local farmers using cuttings from the Farm; the seedlings will be purchased by the Farm and planted out next year and surplus seedlings may be sold to other farmers. Plants were being trimmed and parts not being planted were being dried on the road for sale as leaves.

After the Farm inspection we drove 270km back to Nanjing (3+ hours) and went straight to the Zhongshan Botanical Gardens at Nanjing and met the Director, Mr Huang, who introduced us to Ms Huang Su Zhen, a botanist and plant breeder at the institute. They have had a stevia breeding program since 1976, their current aim is to develop varieties for Eastern and Southern China which can be grown from seed as well as cuttings – even though seed propagation loses purity after three or four generations. The aim is for high R-A types with total steviosides of 18% and with 80% R-A. This hybridization program has two varieties ready for release – Zhongshan 2 and Zhongshan 3, which should set seed in the south but not in the north. Expected leaf yields are 220-250 kg/mu.

Ms Huang speaks Japanese but not English and is collaborating with a Canadian company in her research. We inspected a seedling nursery established from cuttings and a trial seed crop of mixed lines, for seed viability evaluation, which was starting to flower. We were offered seed samples of various lines for evaluation when seed is available (after November). The north of China need seed grown varieties to reduce their costs; southern areas need day neutral lines which will ratoon. (I'm not sure if Southern China means just south of 38°N, Nanjing-32°N or further south). Throughout the visit to Nantong and the Botanical Gardens Xu Gang was interpreter and managed the technicalities of stevia very well indeed.

From Botanical Gardens taken to bus station and travelled 100km to Yangzhou, arriving 8pm.

Friday 21st – organized travel and money.

Saturday 22nd – travelled by bus through Jiangsu Province and south-west Shandong Province to Qufu. An interesting look at farmlands, including some back roads – 11 hours, 570 km.

Monday 24th – short trip (40 km) by bus to Jining. Was met by Zhang Jingong, Trade Department of Shandong Huaxian Stevia Company and drove across town to the company office and factory. Received a very warm welcome from the Chairman (and major shareholder), Zheng Shu Wang, and other senior staff, including Ding Xin Jian (General Manager) and Wang Wei Jun (Marketing Manager). We had a long discussion in the board room with Zhang interpreting (later a second Trade Department Rep also assisted with interpreting and later came to the lunch the Chairman hosted).

Huaxian Stevia is the largest and oldest stevia factory in China and produces about 50% of China's total production, i.e. about 1,000 tonnes out of 2,000 (this might be changing with new factories in the north). The main factory was built in the early '80s but some equipment looks older. Fifty percent of production is sold within China, 40% to Japan, the rest spread around, including Korea, Indonesia and also USA. Sales to USA were twenty tonnes in the last twelve months, for use as dietary supplements; they hope use in foods will occur in the near future and so increase demand.

They are accredited as a 'Green Food' (a national program) and as a Natural Product. They are ISO 9002 Quality Assured and Kosher and Hal-al approved. In discussing health and safety aspects they agreed to provide copies of some of the articles and papers submitted to Chinese authorities to gain natural food status for addition to foods – anything to help open markets. They also referred to an international symposium on stevioside held in Japan on June 6th, 1984, as a significant event in obtaining approvals.

They assist farmers in growing new varieties by providing some seed but usually vegetative cuttings. Material usually originates from the National Agricultural Science Institute, Beijing or from Japan. They offered to send us some seed samples for evaluation when available later in the year. Their newest variety has 14% R-A and 17.5% total stevioside. Farmers don't get paid any extra for growing high stevioside content leaves. Incoming leaves are not routinely analysed for content so they do not know the actual leaf contents produced or the factory recovery rate (they assume it to be 90-95%).

The company produces six stevioside sugars, including two high R-A grades. They don't believe a water only extraction process (no methanol) can produce a high quality product. They have recently released a low sweetness product (100 times sugar), being a blend with manitol and dextrose.

We inspected some growing crops of plants kept after a harvest of leaves for seed production – the leaves were stripped and the stems left intact (these seed crops were not as impressive as the crops seen later at Zibo). We also inspected the factory. Dried leaves are stored for year round factory

production – up to 5,000 tonnes is stored in sheds in mesh wrapped bales like two-thirds sized cotton bales. Initial extraction is in a series of large open steel tanks where the leaves are stirred with warm to hot water with mixing paddles. They undertake about seven washings on a batch (not continuous) process. The crude liquid is then filtered before concentrating in heated vessels. Concentrate is then put through resin extraction and ion exchange before final crystallization. Final product is a powder (ground crystals). They don't sell any liquid products as liquids can only be stored for about 35 days when shipping. The laboratory was quite small with an HPLC unit and little else. All batches (10 per day) of product are analysed for stevioside-A, rebaudioside-A, rebaudioside-C and dulucoside-A as well as ash, moisture, metals etc. For calibrating the HPLC unit they obtain reference samples from UNICOOPJAPAN, Shinichi Shibaskii (e-mail: sibasa-s@unicoop.co.jp) – the last samples were 94.5% St (40 grams) and 95.4% R-A (50 grams).

I discussed NIRS with them as an alternative to HPLC. They were extremely interested. If it can be fairly portable for leaf analysis, the reaction was "when can we have one". They are interested in co-operation to develop this. (Since returning to Australia I have received a phone call and an e-mail reminding me that they want more information on NIRS.)

The price for stevioside is currently very low (under US$15,000/tonne), pushed down by Japan and the "Asian crisis"; excess production, especially in northern China has contributed. With low prices leaf production is reducing. Prices will go up again, although the past highs of US$60,000/tonne were excessive. North China produces higher yields of 3-400 kg/mu compared with 2-300 kg/mu in central areas.

They have been involved in two overseas joint venture proposals which fell through – Vietnam and Uruguay. They would consider some involvement in processing in Australia; China make very cheap factories and equipment. They suggested that unless we are involved in the selling of the stevioside we won't make much profit in Australia; growing will be expensive here.

They were not aware of any stevia production at Nantong for the Japanese or of stevia breeding at the Nanjing Botanical Garden. Before leaving I asked if they knew of an Agricultural Science Research Institute/Sweet Chrysanthemum Study Centre at Zibo. They did not think there was any stevia grown there and knew nothing of the organization. Zhang did offer to ring the number I had to find out what they did and if it was worth visiting. He did not get through that day and I rang him next day when he said that I should visit them and gave me a contact etc. It would appear that he also convinced them that they should talk to me.

After this extensive discussion and inspection they (Zhang) drove me back to the hotel in Qufu.

Tuesday 25th – travelled to Jinan.

Wednesday 26th – contacted Mr Feng Xiao Hong, Zibo in the morning to arrange a visit for Thursday. Afternoon – received a message from Zibo requesting a visit that day. He drove from Zibo (100 km) with an interpreter (Ms Wang Lu Xia, a highschool teacher) to meet me at Jinan and insisted it was important enough to return to Zibo with him that evening! We had a long discussion that evening about stevia.

Thursday 27th – met with Mr Feng and the head of the Institute at their office. Also present was Mr Wang Jian Zhong, a very competent officer from the Zibo Municipal Foreign Affairs Office, acting as interpreter. The Zibo Agricultural Science Research Institute is a municipal department of agricultural research and extension covering the main crops of the region. One of their major activities is to produce, on their own land and on farmers' land, high quality seed of improved

varieties for most of their crops, including many vegetables. As well as selling seed to farmers they also sell other inputs such as fertilizers and sprays.

Stevia growing in the area is based on the production of seed for planting elsewhere, especially in North China but also for export markets – recently they sold 100 kg of stevia seed to Spain (enough for planting more than 600 ha). Leaves from the stevia seed crops are also harvested and exported to Korea. More recently (for two years), Korea have also been taking some stevia stems (Zibo suggested it was for stock feed but presumably it is to extract other compounds). The Institute buys the leaves from the farmers and exports them on their own account. For the farmers, seed can be a more important income than the leaves. The latest varieties to be grown for seed originated from Japan but in the past they have also operated their own selection and breeding program. Korea has recently opened a joint venture factory near Harbin with all stevioside output going to Korea (brochure). There is no leaf analysis carried out at Zibo but they are sure the varieties are high R-A types – (presumably their own selection work was mainly based on leaf yield).

Farmers can harvest up to 25 kg of seed per mu (375 kg/ha) – far in excess of any other reported seed yields. We inspected some seed crops approaching harvest and I can believe this yield potential. To harvest, every one or two days the farmers cover the seed heads with a bag and shake off the mature seed. In this area, Zibo are adamant that the second year crops have lower leaf yields and so they don't ratoon any crops (maybe if the plants are let go to seed, this is the case). In one seed crop, there was evidence of a root disease but this was the fourth crop in a row on the same land. If rotated each two to three years, diseases are said not to be a problem. Seed quality is 80% minimum germination up to six months from harvest. After this, germination drops away quickly and eight months is the limit of reasonable performance. This could impose some problems for Australia if we plant out in August. We would need to obtain late harvested seed (December?) to plant in a nursery in June. There is an average of 500,000 seeds per jin (500 gms), i.e. one million per kilogram. Recommended planting density is 10 cm x 20 cm rows or 15,000 per mu.!! Leaf yields can be up to 400 kg per mu (6 tonnes/ha); this may be for their seed planted in North China. Harvest of leaves is by stripping individual leaves two or three times rather than cutting stems.

There are four or five varieties being grown for seed. These include 99-81 and 83-27 from the local selection program (99 and 83 are the years) and Lee and Toyota 2 and Toyota 4 from Japan. Variety differences include height, leaf size, leaf shape and leaf margins. They suggest there is a direct correlation between leaf size and leaf yield.

Amongst other work at the Institute is a large wheat breeding program aimed at improving the performance under moisture stress and lower rainfall (for non-irrigated rising areas).
Mr. Feng, the stevia specialist is also a wheat breeder. I commented that in Australia much of the wheat research and breeding is also aimed at improving water use efficiency and tolerating moisture stress. Maybe this is an area where we could exchange notes etc. There is a lot of technical knowledge within the Institute, yet they were totally unheard of at all the other stevia centres visited.

After the farm inspections and lunch, hosted by Zibo Municipality representatives, returned to Jinan by bus.

Friday & Saturday 28th, 29th – Returned through rural areas by buses to Shanghai via Linyi, Huaiyin and Yangzhou (870 km).

Monday 1st October – early plane to Singapore and Australia.

4.2 Trip report to Japan

D.J. MIDMORE
TRIP REPORT, 5-26 JULY 2001

After lunch at JICA I met one old friend (Mr Yochiro Eguchi) and three persons with close interest in the stevia industry: Dr Tetsuya Sumida, Consultant from Stevia and Advanced Agricultural Development; Mr Kunio Kakegawa of Maruzen Pharmaceuticals Co Ltd and Dr Tadashi Katabami, Senior Manager, Food Staff Development of Dainippon Ink and Chemicals. Mr Kakegawa gave me a recently published book on stevia, in Japanese, to which Dr Sumida had contributed one long chapter. Between them they offered much information on stevia, although they were reluctant to (a) offer germplasm, (b) let me know about current field sites in Japan. They claimed that all stevia production now takes place in PRC (and that may be true for their companies). Apparently, above a line of approximately 38 N, in Japan stevia used to be replanted every year, and below that line it was planted and then ratooned for 3 or 4 years, with annual sequence of yields of 3, 5, 6, 4 t ha^{-1} dried leaf. Vegetative cuttings (no need for rooting hormone, but better if with it) were used as planting materials, making sure that they are kept out of the wind and preferably at 20 C (for an earlier start in the field) and without drought stress (should show no signs of wilting). Dr Katabami talked of the large expansion in PRC, originally in S. China but now largely moved to Manchuria where seedlings are more important than cuttings for a February sowing and May transplanting. Apparently the LD in the N are more desirable, inhibiting flowering of this SD species. Knowledge of likely flowering is important in Australian environments, so that harvest can take place prior to flowering, so that regrowth of vegetative parts can continue. The need to recut through the season limits yield potential, hence the move northwards. Traditional production in the S. island of Okinawa in Japan required x 3 harvests to avoid flowering, and even in Shanghai flowering occurs in August and then 2 months later if plants are not cut back. But, as plants age stevioside concentration declines, so a management dilemma! When asked about day neutral types, they referred me to the Canadian Royal Sweet Technology Company (can't find them on the www) who have such types. In the first crop, plants reach c .1.0 m, and in the second crop c.1.5 m Rainfall/irrigation should provide c. 100 mm $month^{-1}$.

As a diploid species the earliest approach to variety selection is through selection of individuals, and vegetative propagation. Plants are self incompatible (I'm not sure of pollination mechanism, wind? insect?), and in Machuria farmers are collecting segregating populations of seed for replanting in the following year.

Talking about quality, the normal stevioside:rebaudioside ratio is 1.0:0.4 but they have three varieties with reversed ratios that they are applying for PBR with MAFF (but why, if not growing?) They are:

1. Hotien (1:2)
2. Seiten (1:5)
3. Shyuten (1:10)

The higher rebaudioside is favoured because it has less associated taste. I primed them about food safety, and we had a useful and positive discussion. In Japan stevia is considered as a natural product, and under fundamental law it doesn't need toxicological studies to support its use in diets. However, the real responsibility for safe use in now with the manufacturers. Stevia was a member of the GRASS group of compounds in Japan (the Grant Recognised Association… Safety – includes products such as sugar) but recently exposed to a new law which requires the companies using it to prove that it has no toxicological effect.

Stevia containing four major compounds (R-A, R-C) in various proportions (acc to variety – they say they have no data on environment or g*e effects on the ratios of compounds – but also mention the use of enzymes to raise rebaudioside content) so it is difficult to examine toxicologically all combinations. Katabami says that the chemical composition issue is now resolved, and they are looking at the four substances/ compounds that are converted to steviosides at the end of the human digestive tract – and checking to see if steviole is carcinogenic.

They will publish their results in the Public Record in early 2002, and in the Codex Alimentarius [In English, Latin?]. We must check beforehand with FSANZ to see whether acceptable for registration of steviosides in Australia?

On the issue of varieties, they say that "conventional" varieties are kept at the Japanese Genebank (and again said no stevia grown in Japan – my host said that they had privately told him that the station 60 km from Tokyo where I was to have visited had poor growth of stevia, and that they were embarrassed of that). I will apply officially for seed (but not sure of likely outcome) in a couple of weeks.

We changed the conversation to China, to extraction and processing. I asked for contact around Shanghai (for Andrew Rank's visit) but they said they couldn't recommend any because growing is "speculative" and moves around according to opportunity. Approximately 10 companies import crude powder from PRC to Japan. The powder is extracted at 50-60 C (x 2 or x 3 extractions) and refined in Japan, purification using resins that are specific for the main components. The compounds are released from the resins using alcohol (methanol) and 80% are removed in the first extraction. They are then passed along a +ve/-ve ion exchange to clear of colour impurities (as for sugar).

After the ion exchange, through vacuum pumping the concentration is raised to 30-40%. Drying is done with a spray drier, and the water content in final product is 3-<5%.

The leaf product imported should contain <10% moisture, and <5% dead leaf, flowers, stems or petioles. There is no payment based on quality. For harvest, stems/branches are cut and hung to dry, after which they are shaken to remove the leaves. Asked what Australia should do to ensure a viable industry, Dr Sumida said pay attention to the needs of the growers – physiology, nutrient water and photoperiod requirements, while Dr Katabami said we should ensure that production was matched with marketing requirements (and to promote sugar as a biofuel – so more demand for sweetener!) and Dr Kakegawa spoke of the competition with China at US $20/kg for 80% purity raw product. They emphasised the evolution in reduction of costs, showing how their production base had moved from Korea ➔Taiwan➔Indonesia➔PRC in efforts to reduce the cost of raw materials (and raise their own profits?!)….

4.3 FSANZ Position on Steviosides

FSANZ INFORMATION SHEET

STEVIA AND STEVIOSIDE

Stevia rebaudiana is an herb belonging to the chrysanthemum family which grows wild as a small shrub in parts of Paraquay and Brazil. The leaves have traditionally been used as a sweetener. The leaves of *Stevia rebaudiana* contain 8 different steviol glycosides, the major constituent being stevioside (triglucosylated steviol), constituting 5-10% in dry leaves. Besides stevioside, rebuaudiosides A-E and dulcoside A and B are the other sweet components of *Stevia rebaudiana*.

Stevioside which is extracted from *Stevia rebaudiana* is a high intensity sweetener, 250-300 times sweeter than sucrose, which has been used for several years in countries such as Japan as a sweetener in a range of food products.

The stevia herb and the ground-up leaf of the herb can be sold in Australia and in New Zealand as *Stevia rebuadiana* as it is not prohibited under Standard A12-Metals and Contaminants in Food of the Australian Food Standards Code or Standard 1.4.4 – Prohibited and Restricted Plants and Fungi of the Joint Australia New Zealand Food Standards Code.

Public Health and Safety Considerations

Stevia

Some published reports have raised concerns about the safety of this herb, specifically in relation to potential reproductive effects. A publication by Planas & Kuc (1968) links stevia use with contraceptive effects in Paraguayan Indian women, and provides the results of a study in female rats which indicates a decrease in fertility following treatment with stevia leaf extract. Subsequent attempts to repeat this result were unsuccessful with both stevia extracts and with highly purified stevioside (reviewed in Nabors and Gelardi 1991).

A more recent study by Melis (1999) has reported that a concentrated aqueous extract of stevia induced changes in the testes and in hormone levels that are consistent with the possibility that stevia may decrease fertility in male rats. On the other hand, reproductive studies in rats and hamsters using relatively pure stevioside did not demonstrate any reproductive effects. Thus, while there are still unanswered concerns regarding the safety of stevia and stevia extract from these aspects, these concerns do not appear to be related to purified stevioside.

Stevioside

The toxicity data on stevioside was considered in 1999 by the Joint (FAO/WHO) Expert Technical Committee on Food Additives (JECFA), an international committee established to assess the safety of food additives. JECFA prepared an extensive evaluation report on the available safety data on stevioside but was unable to recommend an acceptable daily intake (ADI) because of some deficiencies in the data. Firstly, there was lack of human metabolism studies on stevioside and steviol. Secondly, the potential mutagenicity of steviol had not been sufficiently tested, particularly in vivo.

FSANZ re-visited the available toxicity data on stevioside following receipt of an application (A397) in August 1999 to permit the use of a 98% pure extract of stevioside as a food additive. In particular, FSANZ examined in detail the issues/critical studies that caused some concern to JECFA. FSANZ supported the conclusions of JECFA that the available data is insufficient to ensure the safe use of stevioside. In order to resolve the outstanding safety issues, FSANZ requested the following studies from the applicant:

Human metabolism data on stevioside and steviol; and
In vivo mutagenicity data on steviol.

The Applicant could not supply this data and the application was subsequently withdrawn on 28 June 2000.

Should stevia be considered a novel food?

The new Novel Food Standard (Standard A19 of the Australian Food Standards Code or Standard 1.5.1 of the joint Australia New Zealand Food Standards Code) provides the opportunity for the safety of the herb, *Stevia rebuadiana*, to be re-visited . This question was considered in consultation with the State, Territory and New Zealand Senior Food Officers in September 2000. On the basis that there is no consistent evidence of a health concern in humans, and that the use of ground-up leaves of stevia in food would be self-limiting, it was agreed that Stevia should not be considered a novel food. FSANZ will, however, continue to monitor the safety data on Stevia.

FSANZ's current position on stevia, stevioside and extracts of Stevia

Stevia

Stevia (whole leaf, crushed leaf) is considered to be a food and therefore can be legally sold in Australia and New Zealand.

Stevioside and extracts of stevia

Stevioside (or a concentrated extract of stevia) is considered to be an intense sweetener. There is no permission in either Volume 1 (The Australian Food Standards Code) or Volume 2 (the joint Australia New Zealand Food Standards Code) to use or sell stevioside in Australia or New Zealand. A plant extract is considered to be a food additive if it fulfils a technological function normally associated with a food additive, eg, an intense sweetening function.

Prepared 9 August 2001

References

Planas GM and Kuc J (1968) Contraceptive properties of *Stevia rebuaudina*. Science, 162, 1007.

Nabors LO and Gelardi RC (1991) Alternative Sweeteners, Calorie Control Council, Marcel Dekker Inc.2nd Edition (pp295-307).

Melis MS (1999) Effects of chronic administration of *Stevia rebaudiana* on fertility in rats. Journal of Ethno pharmacology, 167, 157-161.

4.4 Toxicology

Guidelines for establishing the toxicological profile of food additives, processing aids, contaminants and packaging material

Edition JULY 1998

The principles the Australia New Zealand Food Authority employs when evaluating toxicological data are in accord with those outlined by the Joint Expert Committee on Food Additives (JECFA). These Guidelines are therefore intended to provide guidance rather than to detail specific requirements.

Introduction

Toxicity testing is a major platform upon which to assess possible public health risks from exposure to chemicals. It is, however, an evolving science. At the earliest stages of its development, test protocols reflected the rudimentary knowledge of the mechanisms by which chemicals could exert harmful effects on living organisms. Research and technological advancements have resulted in a better understanding of the biochemical, physiological and pathological bases underlying these effects. Accordingly, toxicity testing standards and protocols have become more comprehensive and precise, while interpretation of the data has become more sophisticated.

Inevitably, in conjunction with the growing public demand for reassurance, this sophistication has created a demand for more and more extensive testing. Debate over what constitutes an adequate package of chemical safety data for regulatory purposes continues.

While it is recognised that resource limitations and economic factors place some constraint on what may be feasible in a toxicological profile, these should never be allowed to compromise safety. There should always be scope for reasoned debate over the interpretation of a toxicological profile. Toxicity tests should be designed, conducted and interpreted according to acceptable contemporary methods.

To help investigators in this respect and to allow them to fully use their knowledge of the properties of the chemical they are assessing, the toxicological profile and study design are outlined in general rather than specific terms. Investigators are expected to use the consequent freedom to provide a clear insight into the toxicity of a test material.

1. General instructions

Data should be substantially complete (as at the time of your application) and be well organised.

Data should be presented in detail sufficient to allow independent scientific assessment (for example, you should provide individual animal data).

You should supply copies of original reports. Summaries and reprints of published material do not usually contain adequate detail.

You must submit all information in English. Where published material is not originally in English, you should supply the original language version and an English translation.

You should include a complete inventory of available toxicological data. This inventory must indicate any studies which you may have provided with earlier applications or submissions.

Details of studies planned or in progress should be disclosed, together with their projected completion dates. If your application includes details of interim studies, the final report, together with any interim reports, should be submitted as soon they become available.

Irrespective of the successful outcome of any application, any additional studies relevant to the safety assessment of the chemical should be provided as soon as they become available.

You should include a summary which concisely deals with every aspect of toxicity studied. Normally, the summary should not extend beyond a few pages. Tables are a good way to condense data. Studies reported in the summary should be cross referenced with reports in the main submission.

You must disclose details of any applications to other regulatory agencies—state the outcome of such applications and whether any of the data was rejected, either in the submission or by notification of the Authority when the outcome is known. Where an application does contain data previously rejected by another agency, an explanation as to why the rejection was not considered valid should be submitted. You must not omit any report which could influence assessment of safety of the substance.

All studies should be conducted in accordance with an acceptable code of good laboratory practice (GLP). All studies conducted in Australia should be in accordance with the *Code of practice for the care and use of animals for experimental purposes.* 2 The report must include certification of compliance in the conduct of each study.

Each study should clearly identify the name and address of the performing laboratory, the names of the responsible scientists, the report code number and the dates the studies were performed.

You must specify the purity and batch number of the material used in each test. It may be appropriate to cross reference with data in the chemical profile. Keynote studies, from which a no observable effect level (NOEL) is established, should be undertaken with material of a comparable composition to that intended for food use (that is, commercial grade rather than highly purified material).

Study details should include:

the route of administration;

the dose levels;

the number of animals per dose level;

the animals' origin, sex, weight range and maturity;

the frequency at which observations were made;

the duration of each study;

the relationship between the time of administration and the onset of the effects observed; and

all measurements made.

In the summary you should identify all compound-related biochemical and physical changes observed in the study with appropriate cross-referencing to the detailed data. Where it is claimed that the manifestations are not toxicologically significant (for example, minor changes in organ weight), evidence of their reversibility may be required. In anticipation of such a possibility, it may be possible to include sub-groups for subsequent recovery assessment from earlier tests.

You should include detailed results from individual animals in prolonged toxicity studies. Where they will help review the data, you should include supplementary tables or diagrams (for example, growth curves, tumour incidence tables). It should be possible to organise tables so that the most appropriate comparisons (for example, control and treated groups) are contained on the same page and results of histopathological observations can be readily evaluated in relation to dose, sex and duration of treatment.

2. Toxicity tests

2.1 Acute toxicity studies

The degree of hazard presented by chemicals depends on many complex factors. The effects of a single large dose of a toxic chemical may not necessarily reflect risks associated with the low levels to which dietary exposure to a food additive or contact material is ordinarily expected. However, acute studies of systemic toxicity do provide insight into bioavailability, potency comparisons with other known toxic agents and an indication of which target organs might be affected.

Acute oral toxicity studies should be performed in both sexes to assess possible gender-related differences in response. The rat is the preferred animal for acute toxicity studies. Studies using other species are important for revealing possible species differences in response. Since the ultimate goal is trans-species extrapolation to man, knowledge of such species differences may be crucial.

Acute dermal, inhalation and parenteral studies may be useful where the bioavailability is markedly influenced by the route of administration, or where assessment of occupational exposure in food handlers may require consideration.
Skin and eye irritation studies and skin sensitisation studies may also be relevant where the nature of the material suggests likely consumer or occupational exposure (for example, powdered enzyme preparations).

Estimates of the LD50 are not normally required and the limit test procedure is acceptable. Of greater importance is the provision of details of the toxic effects observed, cause of death and other data which would enable assessment of acute toxicity to be more comprehensive.

2.2 Sub-chronic toxicity

Sub-chronic studies of at least 90 days duration are essential to determine the effects of repeated exposure and as a preliminary dose-ranging study before starting chronic studies. Sub-chronic studies should demonstrate a range of activity from the NOEL through to a clearly toxic level. Often this range can be encompassed in a single study using a control and three test groups. The test material is ideally given continuously as an admix in the diet, but unit dosing by gavage or capsule may be appropriate in certain circumstances.

Evidence of the stability of the compound in the feed and the actual dose rates achieved should be given.

Observation on growth, behaviour, food consumption, clinical abnormalities and mortality should be recorded throughout the study. All animals dying during the test should be examined for macroscopic and microscopic changes. At the conclusion of the dosing period, surviving animals (other than those allocated to recovery experiments) should be killed and data recorded on organ weights, gross

morphology and histopathology. Analytical tests such as haematology, blood biochemistry, urinalysis and other biochemical tests should be done, at least at termination, and where sampling would not compromise the study, at earlier intervals. Organs identified as systemic targets in acute toxicity tests should be carefully scrutinised.

Compound-related changes should be clearly stated in the summary with appropriate cross reference to the detailed data. You must bear in mind that the objective of the study is to demonstrate biologically important responses. Where statistical methods are used to support the evaluation of the responses, the validity of the method and the power of the test to establish a compound-related effect should be considered. A statement of the smallest difference which would achieve statistical significance under the conditions of the test would facilitate interpretation.

At the end of the test material exposure period it may be useful to allow some of the test animals to undergo recovery to provide information on the reversibility of the observed changes. The recovery profile is of particular importance if you are claiming that biological responses to the compound are toxicologically insignificant.

2.3 Long-term studies

Long-term studies are particularly important for two reasons:

i. they simulate the effects of lifetime exposure and may reveal toxic effects which appear later than those which are apparent from subchronic studies; and
ii. they permit a comprehensive assessment of the oncogenic potential of a compound.

2.3.1 Chronic toxicity studies

Chronic toxicity studies normally involve long-term continuous daily exposure to graded amounts of the test material in the diet. The use of a rodent and a non-rodent species is desirable to provide data on inter-species variation. The rat, mouse and dog are the species whose toxicological response profiles are best known. Studies in the dog are generally acceptable when limited to only six months.

Chronic toxicity studies should normally include one control and three test groups. The design is similar to that of 90-day studies and the results of those studies may be of particular importance in determining the range of doses for the chronic studies. The highest exposure level should induce a recognisable response. For materials of low intrinsic toxicity, where a response may be difficult to achieve, the highest level should be the maximum which is practicably achievable. At least one exposure level should result in the NOEL. Survival rates in all groups should be high enough to enable a meaningful statistical analysis of the data.

The interpretation of chronic toxicity studies may be greatly influenced by toxicokinetic considerations, particularly when species differences are apparent. Wherever possible, plasma levels of the test compound (and/or its metabolites) should be measured at steady state.

2.3.2 Oncogenicity

Studies, preferably in two species, will be needed to provide an adequate basis for assessing oncogenic potential when:

i. the compound's chemical structure has features in common with compounds of established oncogenic activity;
ii. the compound, or any of it's metabolites, impurities or degradation products, expresses genotoxic activity in an appropriate battery of tests (see section 2.5); and

iii. projected use patterns of the material in food will result in exposure of a significant proportion of the population and it is considered necessary to establish that the compound does not constitute an oncogenic risk.

The design of an oncogenicity study is similar in many ways to that of a chronic toxicity study, however there are significant differences which may compromise the interpretation of combined studies.

In an oncogenic study, there should be at least two exposure levels, and possibly three, where systemic toxicity is not apparent. The upper acceptable exposure level is the maximum tolerated dose (MTD), which should produce no more than 20 per cent impairment of growth and should not significantly alter survival rates (other than by the induction of fatal cancers).

The choice of species and strain is important. A well-defined and stable incidence of neoplasms in untreated controls may be crucial to the determination of whether a particular lesion is compound-related. Historical data describing the normal incidence and variation in tumour rates would be useful, but this will not necessarily resolve conflicts in the assessment. For example, if the control group incidence is below the normal range but the test groups produce an incidence within the historical control range, the strength of any dose-related trend will be of major importance in determining the outcome of the test.

2.3.3 Chronic toxicity/oncogenicity studies

A combined chronic toxicity/oncogenicity study may provide adequate data with which to identify major chronic and oncogenic effects. However, careful design is important to ensure that objectives do not conflict, masking potential results. For example, an exposure level high enough to induce a detectable level of chronic toxicity may confound the detection of treatment induced tumours at termination of the study. Conversely, the MTD may not provide unequivocal evidence of chronic toxicity.

2.4 Reproductive and developmental studies

A well designed multi-generation reproduction study should provide information relevant to the effects of a substance on all aspects of reproduction, including sexual behaviour, gonadal function, spermatogenic and oestrus cycles, fertility, fecundity, parturition, lactation, pre- and post-natal growth, development and maturation of offspring.

The study may also provide preliminary data on teratogenesis and serve as a guide for further studies. The most practical animals in which to conduct these tests are the rat or the mouse and the study would normally be conducted with a control and three test groups.

Developmental studies are intended to provide information on embryotoxicity, teratogenicity, altered growth and the induction of functional deficits (postnatal behaviour). Indications of maternal toxic responses should be reported as an aid to the interpretation of any effects. The rat and the rabbit are the two species in which toxicological responses have been most characterised.

It is recognised that research activity is currently being directed into developing short-term (in vitro) alternatives to these more conventional tests. These tests may be useful for screening purposes, however, they have not, as yet, been validated for regulatory purposes.

2.5 Genotoxicity studies

To determine the potential for a compound to contribute to genetic damage in humans, it is essential to conduct mutagenicity studies which detect different kinds of genetic endpoints in phylogenetically different organisms.

A basic package of genotoxicity studies will generally comprise:

i. a test designed to demonstrate the induction of point mutations (base-pair substitution and frame shift) in a microbial assay (for example, salmonella microsome test) with and without the use of appropriate metabolic activation systems;
ii. a test designed to demonstrate the production of chromosome damage in an in vitro mammalian cell assay (for example, chromosomal aberration assay in Chinese hamster ovary cells) with and without the use of appropriate metabolic activation systems;

If a positive result is returned in either test, the following tests should also be undertaken:

iii a test designed to demonstrate the production of cytogenetic damage (for example, micronuclei) in the bone marrow or other proliferative cells of intact animals; and
iv a test designed to demonstrate genotoxic damage involving other than cytogenetic damage (for example, UDS or P32 post-labelling adduct formation) and preferably a suspect or known target tissue for the chemical substance.

Supplementary, tests (for example, sister chromatid exchange, micronucleus test) should also be used to provide clarification of unexpected or equivocal results in the basic test portfolio, or to provide additional evidence. In vitro germ cell tests using laboratory animals (for example, mouse-specific locus tests heritable translocation assay) could be essential for the evaluation of a suspected mammalian mutagen.
Since this is a rapidly developing field, relevant and validated tests representing other genetic endpoints (for example, aneuploidy) should be used to provide supporting information as they become available.

2.6 Additional studies

2.6.1 Toxicity of metabolites, degradation products and impurities

Although it is recognised that toxicity tests usually determine the toxicity of the principle compound under evaluation, impurities, degradation products and metabolites may sometimes make an overall contribution to safety assessment. Generally speaking studies using the manufacturing grade of the compound provide an estimate of the potential toxicity of the parent compound as well as the principle metabolites and impurities. However, in certain cases specific toxicological information on the individual components may be useful. When food processing or chemical degradation results in exposure, via food, to derivatives of the chemical under evaluation, their contribution to the toxicological profile may also warrant consideration.

2.6.2 Potential for intolerance and other adverse sensitivity effects

Intolerance to food additives should always be considered a possibility, even though tests for reactions to food additives are not part of the normal data package required for assessment of new substance. Satisfactory animal models to predict food intolerance in humans have not been developed and interpretation from human studies may present difficulties. Where there are grounds to anticipate or suspect food intolerance reactions, reasonable studies might include double blind challenge feeding studies.

2.6.3 Nutritional considerations

The use of a food additive which may significantly alter nutritional intake (for example, in synthetic or novel foods) implies a need for nutritional considerations in the interpretation of toxicological

profiles. In appropriate cases, therefore, the relationship between toxicity and nutritional status should be assessed.

2.7 Toxicokinetics and metabolism

Studies on the fate of a chemical in exposed animals add confidence to the extrapolation of toxicological data to humans. Appropriate studies will provide information on absorption following oral administration, tissue distribution and storage, bio-accumulation, bio-transformation and the mode and extent of excretion or elimination of the parent material and its degradation products.

2.8 Human data

You have a responsibility to alert regulatory authorities to any adverse effects recorded in humans associated with any chemical under evaluation. Such data sometimes results from an accidental poisoning or as the result of experience with occupational exposure.

With food additives, it is sometimes practical to conduct well controlled studies in humans using dose rates comparable to, or even exceeding, expected consumption from food. Such studies should be subjected to rigorous ethics review procedures. Their value, in conjunction with animal toxicity studies, lies in their ability to provide highly relevant data on tolerability, particularly with respect to effects which would not be readily detected in animals (for example, gastric intolerance, CNS disturbances etc).

2.9 No observable effect level

The NOEL is the highest dose-level which produces no observable toxic effect in the most sensitive test species. It is expressed in milligrams of chemical per kilogram of body weight per day (mg/kg bw/day).

In a feeding study it may be derived from the concentration in the diet (usually in ppm) multiplied by the average food intake. Where chemical instability in the food is suspected to be a problem, it is essential that feed be prepared and analysed at frequent intervals.

2.10 Acceptable daily intake

The ADI is the daily intake of a chemical which, during an entire lifetime, appears to be without appreciable risk on the basis of the available information at the time. It is derived from the NOEL and is expressed in milligrams of the chemical per kilogram of body weight per day (mg/kg bw/day).

It is accepted that absolute safety of chemicals to humans cannot be established because, whilst one can prove that a chemical can produce a toxicological effect, it is not possible to determine the absolute absence of a toxicological effect.
For this purpose, 'without appreciable risk' is taken to mean that adverse effects will not result even after a lifetime of exposure. Furthermore, for a food constituent, the ADI is intended to indicate the maximum amount that can be taken daily in the diet 'without appreciable risk' to the consumer. Accordingly, as far as possible, this figure is derived from feeding studies in animals.

The ADI for humans is usually calculated as a fraction (normally one hundredth) of the NOEL in the most sensitive species. It is therefore a conservative estimate, taking into consideration possibly unknown species differences in metabolism and toxic potency for humans, and the fact that the human phenotype is more diverse than the inbred strains which are used in animal toxicity tests.