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The Nordic Food Policy Co-operation
The Nordic Committee of Senior Officials for Food Issues is concerned with basic Food Policy issues relating to food and nutrition, food toxicology and food microbiology, risk evaluation, food control and food legislation. The co-operation aims at protection of the health of the consumer, common utilisation of professional and administrative resources and at Nordic and international developments in this field.

The Nordic Council of Ministers
was established in 1971. It submits proposals on cooperation between the governments of the five Nordic countries to the Nordic Council, implements the Council's recommendations and reports on results, while directing the work carried out in the targeted areas. The Prime Ministers of the five Nordic countries assume overall responsibility for the cooperation measures, which are co-ordinated by the ministers for cooperation and the Nordic Cooperation committee. The composition of the Council of Ministers varies, depending on the nature of the issue to be treated.

The Nordic Council
was formed in 1952 to promote cooperation between the parliaments and governments of Denmark, Iceland, Norway and Sweden. Finland joined in 1955. At the sessions held by the Council, representatives from the Faroe Islands and Greenland form part of the Danish delegation, while Åland is represented on the Finnish delegation. The Council consists of 87 elected members – all of whom are members of parliament. The Nordic Council takes initiatives, acts in a consultative capacity and monitors cooperation measures. The Council operates via its institutions: the Plenary Assembly, the Presidium and standing committees.

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PREFACE

The Nordic Committee of Senior Officials for Food Issues is an advisory body of the Nordic Council of Ministers which co-ordinates Nordic work in the field of food and nutrition. The Committee has given the Nordic Working Group on Food Toxicology and Risk Evaluation (NNT) the responsibility to promote co-operation and co-ordination among Nordic countries in matters relating to food toxicology and risk assessment.

Assessment of health risks connected with exposure to naturally occurring toxicants in foodstuffs has become an important area for NNT in the recent years. A series of Nordic reports based on the work performed by the Nordic project group on inherent natural toxicants in food plants and mushrooms has been published:

Gry, J. and Pilegaard, K. (1991) Hydrazines in the Cultivated Mushroom (*Agaricus bisporus*). Vår Föda 43;Supplement 1.

Uggla, A. and Busk, L. (1992) Ethyl carbamate (urethane) in alcoholic beverages and foodstuffs - A Nordic View. Nordiske Seminar- og Arbejdsrapporter 1992:570.

Størmer, F.C., Reistad, R. and Alexander, J. (1993) Adverse health effects of glycyrrhizic acid in licorice. A risk assessment. Nordiske Seminar- og Arbejdsrapporter 1993:526.

Andersson, C., Slanina, P. and Koponen, A. (1995) Hydrazones in the false morel. TemaNord 1995:561.

Søborg, I., Andersson, C. and Gry, J. (1996) Furocoumarins in Plant Food - exposure, biological properties, risk assessment and recommendations. TemaNord 1996:600.

Gry, J. and Andersson, H.C. (1998) Nordic seminar on phenylhydrazines in the Cultivated Mushroom (*Agaricus bisporus*). TemaNord 1998:539.

Andersson, H.C. (1999) Glycoalkaloids in tomatoes, eggplants, pepper and two Solanum species growing wild in the Nordic countries. TemaNord 1999:599.

Andersson, H.C. (2002) Calystegine alkaloids in Solanaceous food plants. TemaNord 2002:513.

The present report aims to: (i) summarise the data on occurence of nicotine in Solanaceous plant foods; (ii) discuss whether the demonstrated occurrence of nicotine is inherent in these plant foods, and (iii) estimate the potential risk from the dietary exposure to nicotine from these foods.

Literature about nicotine in Solanaceous food plants was identified by searches in PubMed, Toxline Plus and Food Science Technology Abstract up to 30 September 2001, using nicotine, nornicotine, anabasine, anatabine and tobacco alkaloids as search terms in combination with Solanaceae, potato, tomato, egg plant and pepper. Literature related to nicotine in tobacco and tobacco smoke was generally excluded. The reference lists of identified publications were screened for additional references not collected in the data search.

The *Project Group* consisted of the following members:

Jørn Gry (co-ordinator)	Danish Veterinary and Food Administration	Denmark
Christer Andersson	National Food Administration	Sweden
Jan Alexander	National Institute of Public Health	Norway
Arne Vidnes	Norwegian Food Control Authority	Norway
Anja Hallikainen	National Food Agency	Finland

The present report has been prepared by Christer Andersson[1], Paula Wennström[1], and Jørn Gry[2] after thorough discussions in the project group, and finally adopted by NNT.

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1. Summary

Nicotine has been detected in potatoes, tomatoes, eggplants, and sweet peppers, all food plants and members of the large family Solanaceae. The nicotine levels were extremely low in fresh potatoes, tomatoes and sweet peppers, below 10 μg/kg. Somewhat higher levels, but still very low amounts, were found in fresh eggplant fruits (up to 100 μg/kg). Processed products contained equivalent or slightly higher levels of nicotine than fresh products (up to 34 μg/kg). No other tobacco alkaloids have been detected in these food plants.

Analysis of endogenously produced nicotine at these low levels requires control of factors that could influence the final level of nicotine in the sample, such as deposition of environmental tobacco smoke during growth, handling or storage, and use of nicotine containing pesticides. The latter possibility seems to be a reality mainly in foods imported from developing countries. Precautions also have to be taken that environmental tobacco smoke does not contaminate the sample during analysis. When the data, as summarised in the present report, are taken together it seems reasonably well established that nicotine does occur naturally in potato, tomato, eggplant and sweet pepper at the low levels reported above.

The average dietary exposure to nicotine from the food plants mentioned above was calculated from information on nicotine content and consumption statistics. The origin of Swedish and Danish consumption data of potatoes and tomatoes are of satisfactory quality, but the intake of eggplant had to be calculated from import/export statistics, and the intake of sweet pepper from less controlled information. The total dietary nicotine intake was found to be on average 1.1 μg/day (88% from potatoes) in Sweden and 1.3 μg/day (70% from potatoes) in Denmark.

Nicotine is very toxic at high doses. The lethal dose in man is 50-100 mg, which approximately corresponds to the nicotine content of tobacco in 5 cigarettes. At lower doses it has many pharmacological effects.

In comparison, the total dietary exposure to nicotine is very low, and seems to be insignificant in relation to exposures giving rise to toxic and/or pharmacological effects.

The dietary exposure to nicotine is about two orders of magnitude lower than the exposure in passive smoking and around three orders of magnitude lower than the direct exposure during cigarette smoking (around 900 - 1 700 μg nicotine is assumed to be absorbed from a single cigarette). In addition to the difference in exposure level, absorption is much lower when exposure occurs in the diet than when by the inhalation route. Absorption from the stomach is poor and 60 to 70 percent of the nicotine is metabolised during the first pass through the liver, whereas absorption in the lungs is good and distributes nicotine systemically. Thus, it seems very unlikely that the low nicotine levels from dietary exposure would cause any toxicological harm in human.

As Solanaseous plants, including some well known food plants, contain nicotine, and the level of tobacco alkaloids in Solanaceous plants can be influenced by genetic modification, it might be advisable to control that the level of nicotine is not significantly altered in genetically modified potatoes, tomatoes, eggplant and pepper fruits.

Poisoning from other tobacco alkaloids than nicotine has been reported, but not in connection with traditional food. For example, anabasine has lead to intoxication in connection to being used to control crop pests and malaria mosquitoes, or by accidental consumption of leaves of the tree tobacco (*Nicotiana glauca*) which contain the compound as the main alkaloid. The nightshade family (Solanaceae) unites 75 genera and over 2000 species, most having their natural habitat in warmer countries. The largest genus in the family is *Solanum*. Some of the nightshade plants are important nutritious plants, like for example the potato, the tomato and the eggplant. Others, like the tobacco plant, is used by man for the production of many tobacco products used as stimulants.

Since Solanaceous plants are particularly rich in alkaloids they have been studied for their content of alkaloids for a long time. The most studied alkaloid is nicotine in the tobacco plant. Because of the close genetic relationship between Solanaceous food plants and the tobacco plant, it seems natural to investigate whether also potatoes, tomatoes, eggplants and sweet peppers have the ability to produce nicotine. These studies have revealed the occurrence of low levels of nicotine in these food plants. The levels detected were so low that it has been questioned whether the occurrence of nicotine in the mentioned food plants might be the result of environmental pollution, or use of nicotine as a pesticide.

The present report aims to summarize the data on occurrence of nicotine in Solanaceous food plants, discuss whether the demonstrated occurrence of nicotine is inherent in the food plants, and estimate the potential risk from the dietary exposure to nicotine from these foods. The report also summarize background information required as a part of the safety assessment required of genetically modified potatoes, tomatoes, eggplants, and sweet peppers.

1. Sammanfattning

Nikotin har påvisats i potatis, tomat, äggplanta och paprika. De är alla livsmedelsproducerande växter och medlemmar av den stora familjen *Solanum*. Nikotinnivån är extremt låg i färsk potatis, tomat och paprika, under 10 μg/kg. Något högre nivåer, men fortfarande låga halter har påvisats i färsk äggplanta (upp till 100 μg/kg). Bearbetade produkter innehåller likvärdiga eller något högra nivåer av nikotin än färska produkter (upp till 34μg/ kg). Inga andra tobaksalkaloider har hittats i dessa vegetabilier.

Analys av så låga nivåer endogent syntetiserad nikotin i vegetabilier kräver kontroll av faktorer som kan påverka den slutgiltiga nivån av nikotin i proven. Sådana faktorer är kontaminering genom nedfall av tobaksrök från miljön under växternas odling, hantering eller lagring, samt användning av bekämpningsmedel som innehåller nikotin. Det senare typen av kontaminering tycks huvudsakligen förekomma i livsmedel importerade från utvecklingsländerna. Förebyggande åtgärder måste även vidtas för att inte tobaksrök skall kontaminera proverna under analys. När de uppgifter som summerats i denna rapport ses i sin helhet kan man fastlägga att det är tämligen väl påvisat att nikotin förekommer naturligt i låga halter i potatis, tomat, äggplanta och paprika.

Medelkonsumentens exponering för nikotin från ovan nämnda livsmedel har beräknats från information om livsmedlens innehåll av ämnet, samt information om hur mycket vi dagligen konsumerar av dessa livsmedel. Konsumtionsdata av potatis och tomat är i Sverige och Danmark av tillfredställande kvalitet, medan konsumtionen av äggplanta är beräknad utifrån statistik på import och/eller export av livsmedlet. Konsumtionen av paprika är beräknad utifrån ännu sämre information. Det totala intaget av nikotin via föda befanns i genomsnitt vara 1.1 μg/dag i Sverige (88 % från potatis) och 1.3 μg/dag (70 % från potatis) i Danmark.

Nikotin är mycket toxiskt i höga doser. Den dödliga dosen är 50 - 100 mg för människa, vilket ungefärligen motsvarar nikotininnehållet i 5 cigaretter. Många olika farmakologiska effekter uppträder vid lägre doser.

Det totala dagsintaget av nikotin via födan är lågt och tycks i relation till den exponering som krävs för att ge upphov till toxiska och/eller farmakologiska effekter vara obetydligt. Exponeringen av nikotin från födan är cirka hundra gånger lägre än den exponering som uppkommer vid passiv rökning och runt tusen gånger lägre än exponeringen vid cigarettrökning (runt 900-1700 μg nikotin förmodas bli absorberad från en enda cigarett). Rökning resulterar inte enbart i en högre exponering för nikotin (via andningsvägarna) än vid konsumtion av nikotininnehållande vegetabilier (och exponering via mag-tarmkanalen), utan absorptionen av alkaloiden är dessutom bättre via andningsvägarna än via mag-tarmkanalen. Via magen är absorptionen förhållandevis låg och 60-70 % av alkaloiden metaboliseras av levern innan produkterna fördelas i kroppen, medan absorptionen av nikotin via lungorna är god och distributionen systematiskt. Därför tycks det osannolikt att den låga exponering för nikotin som uppkommer vid konsumtion av nikotininnehållande livsmedel skulle ge upphov till toxikologiska skador på människa.

Eftersom de växter som behandlats i denna rapport, i likhet med andra växter tillhörande potatisfamiljen, innehåller nikotin och det visats att nivån av tobaksalkaloider i växter kan påverkas genom bioteknologiska metoder, kan det vara lämpligt att kontrollera att nivån av nikotin inte påverkas signifikant när potatis, tomat, äggplanta och paprika genmodifieras.

Förgiftningar av andra tobaksalkaloider än nikotin har rapporterats men inte vid livsmedelskonsumtion. Till exempel har anabasin lett till förgiftning i samband med bekämpning av skadegörare på grödan och malariaframkallande insekter, eller när bladen från trätobak (*Nicotiana glauca*) konsumerats av misstag. Denna växt innehåller osedvanligt höga halter av denna tobaksalkaloid.

2. Introduction

Alkaloids are secondary metabolites occurring in around one quarter of all plants (Lewis and York, 1978). These metabolites are nitrogen containing cyclic bases. The perhaps best known alkaloid is nicotine, the main alkaloid in tobacco (*Nicotiana tabacum*), a plant used for production of many tobacco products used by man as stimulants. Nicotine has a number of pharmacological effects, of which smoking- and snuff-induced dependency on the drug is best known. Minor tobacco alkaloids are nornicotine, anabasine, and anatabine.

The tobacco plant is a member of Solanaceae, the "potato family", which also harbours important food plants such as the potato, tomato, eggplant and sweet pepper. Because of the close genetic relationship between these food plants and the tobacco plant, Solanaceous food plants have been studied for their ability to produce nicotine. Low levels of nicotine have in fact been reported to occur in the potato, tomato, eggplant and sweet pepper. However, it has been questioned whether the occurrence of nicotine in these plant foods might be due to environmental pollution or to residues from the use of the compound as a pesticide (Domino et al., 1993).

The present report aims to summarize the data on occurrence of nicotine in Solanaceous food plants, discuss whether the demonstrated occurrence of nicotine is inherent in the food plants, and estimate the potential risk from the dietary exposure to nicotine from these foods. Further, provided it is concluded that nicotine is formed naturally, this report summarize background information required as a part of the safety assessment of genetically modified potatoes, tomatoes, eggplants, and sweet peppers.

3. Identity and physico-chemical properties

Nicotine was discovered in 1828, but its chemical structure, (S)-3-(1-methyl-2-pyrrolidinyl)pyridine, was not established until the suspected structure (Figure 1) was confirmed by chemical synthesis in 1904 (Kutchan, 1995). The molecular weight is 162.23. Nicotine is a colourless to pale yellow oily base with acrid burning taste (Merck Index, 1996). The compound is very hygroscopic and turns brown on exposure to air or light. It is a steaming volatile capable of forming salts with almost any acid, and double salts with many metals and acids. The pKa-values of nicotine are: pKa_1 6.16, and pKa_2 10,96.

Nicotine (CAS Registry number 54-11-5) is very soluble in alcohol, chloroform, ether, kerosene and oils (Merck Index, 1996). It is miscible with water below 60°C. On mixing nicotine with water, the volume contracts.

3.1. Analytical methods

Early studies on extraction and isolation of tobacco alkaloids were performed by Ukrainian scientists. Methods for efficient extraction of tobacco alkaloids have recently been compared (Jones et al., 2001). The determination of nicotine in food matrixes represents a demanding problem for the analytical chemist. Nicotine is a tertiary base and because most vegetables and fruits are acidic, nicotine is bound to

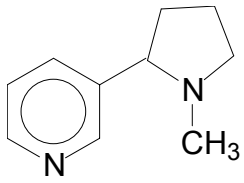

Figure 1. Chemical structure of nicotine ($C_{10}H_{14}N_2$).

the matrix as a salt. Another issue concerning nicotine determination at the low levels found in fruits and vegetables is the potential interference from contamination coming from the chemicals and equipment used, environmental tobacco smoke, as well as from surface contamination of the vegetable/fruit samples (Siegmund et al., 1999b).

A variety of methods for isolation, identification and quantification of nicotine and related compounds in plants and biological tissues have been published. These methods range from early paper chromatographic procedures through gas chromatographic, combined gas chromatographic-mass spectrometric, mass spectrometric, thin-layer, and high-performance chromatographic procedures (Castro and Monji, 1986). Also several radioimmunoassays have been developed for nicotine (Castro and Monji, 1986).

Castro and Monji (1986) were the first to identify nicotine in Solanaceous food plants. These investigators quantified nicotine with a radioimmunoassay. However, Siegmund et al. (1999a) claims the method to be analytically selective but not specific. Furthermore, it requires extensive manipulations and is therefore tedious. Thus, the method is not well suited for studies of a large number of samples (Siegmund et al., 1999a). Below a concentration of 10 μg nicotine per litre, the plasma concentration of nicotine is measured by the radioimmunoassay with a sensitivity less than 90%, whereas the limit of detection in food extracts is less well defined (Castro and Monji, 1986).

Sheen (1988) investigated the nicotine content of Solanaceous food plants using a gas-liquid chromatographic technique having a limit of detection around 1 ppm (=1000 μg/kg) in dry material, which corresponds to approximately 100 μg/kg fresh weight. Davis et al. (1991), Domino et al. (1993), and Siegmund et al. (1999a) used gas chromatography coupled to mass spectrometry to determine the nicotine levels in Solanaceous food plants. In two of these three studies, the limit of detection was 1,0 μg/kg. In the third study, the limit of detection was 0,8 μg/kg (Siegmund et al., 1999a). Considering the low detection limit required for nicotine analysis in food plants, it seems that the gas chromatography-mass spectrometry methods are to be preferred when measuring nicotine in Solanaceous food plants.

4. Biosynthesis

Nicotine is endogenously synthesised in many plant species, but the biosynthesis is most well studied in the tobacco plant probably because of the pronounced accumulation of nicotine in this plant. No investigation on the biosynthesis of

nicotine in food plants has been published. The presentation given here is therefore based to a large extent on data obtained in studies on *Nicotiana tabacum*.

Like all alkaloids, nicotine is formed from amino acids. The pyrrolidine ring (the right ring structure in Figure 1) is formed from the amino acid ornithine trough decarboxylation, methylation, oxidative deamination and ring closure, as shown in Figure 2 (Samuelsson, 1999). The decarboxylation of ornithine is catalysed by *ornithine decarboxylase*. Work on this enzyme has accelerated after the enzyme was isolated from the tobacco plant (Hibi et al., 1994). The product of ornithine decarboxylation, putrescine, is methylated by the enzyme *putrescine N-methyltransferase*. The methyl group comes from methionine. Oxidative deamination of N-methylputrescine is catalysed by *diamine oxidase*, producing N-methyl-γ-aminobutanal. N-methyl-γ-aminobutanal cyclizes spontaneously to the N-methyl-pyrrolinium cation, which reacts with 1,2-dihydropyridine (from nicotinic acid) to yield a product, which is oxidized to nicotine. The pyridine ring of nicotinic acid (the left ring structure of Figure 1), is derived from 3-phosphoglyceraldehyd and aspartic acid (Samuelsson, 1999). Use of labelled nicotinic acid showed that the only hydrogen atom lost was that from carbon-6 and that the pyrrolidine ring system was attached to the carbon atom to which the carbocylic acid group was previously attached.

Although being the main alkaloid in *Nicotiana tabacum*, nicotine does not appear to be the final product of alkaloid production in the tobacco plant. Nornicotine (Figure 3) is produced from nicotine by transmethylation of the N-methyl group. Other alkaloids of *N. tabacum* include anabasine, which is best known as the main alkaloid in the tree tobacco (*Nicotiana glauca*), and anatabine (Figure 3). Various post-harvest reactions that occur in the curing of tobacco leaves result in degradation of

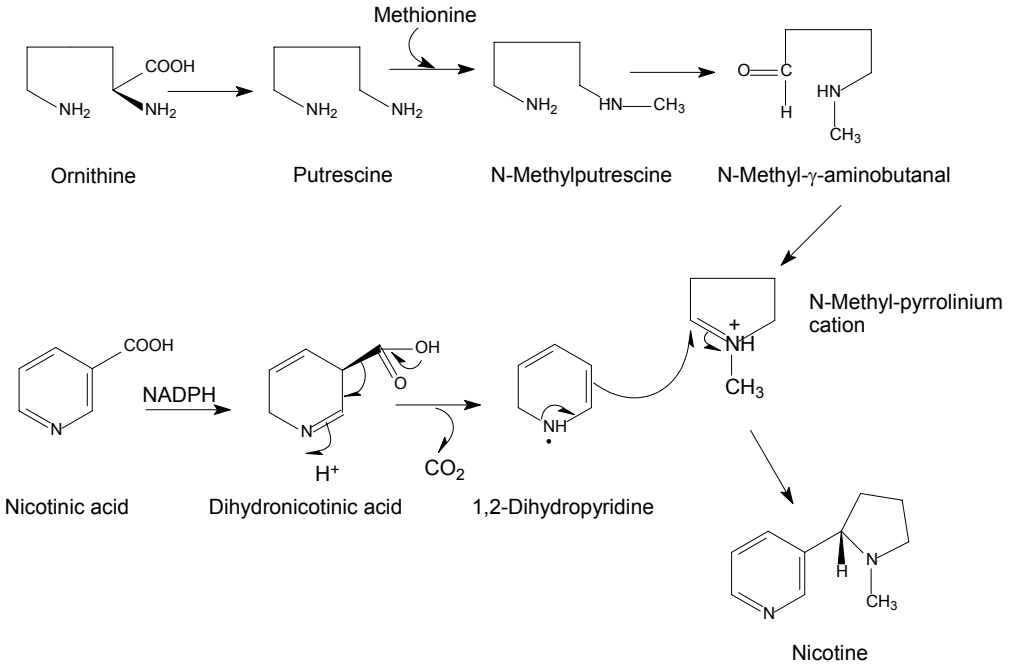

Figure 2. Biosynthesis of nicotine in *Nicotiana tabacum* (after Samuelsson, 1999).

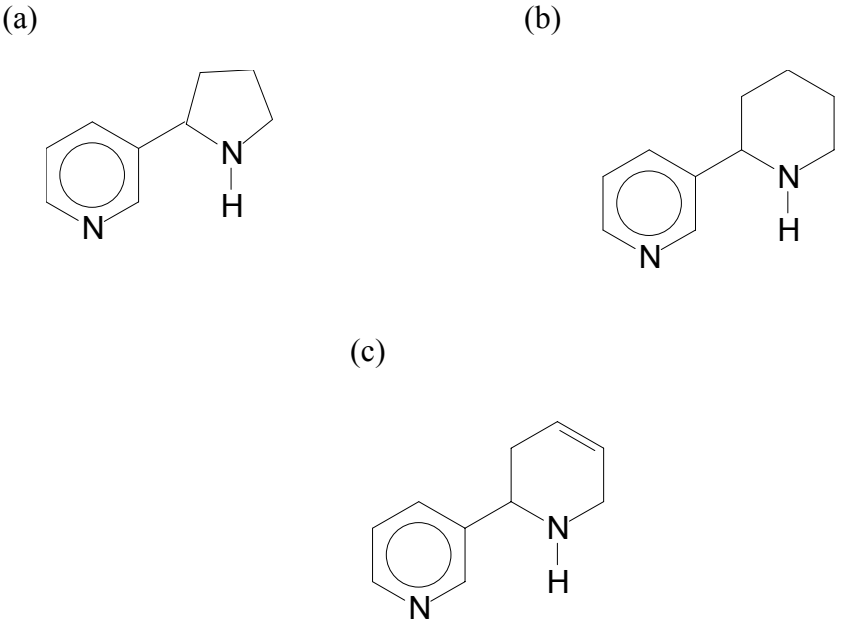

Figure 3. The chemical structure of the tobacco alkaloids (a) nornicotine, (b) anabasine, and (c) anatabine.

nicotine to other alkaloids including its N-oxide, myosmine, and cotinine (Petterson et al., 1991).

Minozhedinova et al. (1978) studied the nicotine metabolism in above ground organs of various wild *Nicotiana* species at the period of vegetative development by supplying ^{14}C-labelled nicotine exogenously. The investigators noted that anabasine is formed in ababasine species of the genus *Nicotiana*, while nornicotine is formed in nornicotine and certain nicotine species of the genus. The low content of tobacco alkaloids in wild species of the genus *Nicotiana* was suggested to be due to the fact that they were able to metabolise the compounds considerably more efficient than the cultivated tobaccos. For example, leaves of *Nicotiana rustica* cultivated in two regions of Turkey, Southeast Anatolia and Ankara, contained 7.71% and 1.17% nicotine, 0.11% and 0.04% nornicotine, and 0.15% and 0.06% anabasine on a dry weight basis, respectively (Kurucu et al., 1998). Most likely, during evolution nicotine accumulators have lost their ability to efficiently metabolise nicotine further.

There has been much speculation about the possible advantage of plants producing these compounds, but little is actually known (Samuelsson, 1999). Most scientists are of the opinion that alkaloids have a role as defence substances in plants against attack by micro-organisms, fungi, bacteria, viruses, insects or other aggressors. Most alkaloids, including nicotine, have a bitter taste and the attacking organisms and herbivores learn by experience to avoid these plants. Support for this opinion is given by studies on hydroponically grown *Nicotiana sylvestris*, showing that (^{15}N)-nitrate is incorporated into nicotine when synthesised *de novo* in response to wounding of leaves (Baldwin and Ohnmeiss, 1994). Another indication that nicotine has a function in plant defence is the observation that nicotine is more abundant in unripe tomato plants than in ripe ones (Castro and Monji, 1986; Siegmund et al., 1999b).

This observation is in accordance with the hypothesis that there is a greater need for defence before the tomato plant is fully developed.

An alternative hypothesis to nicotine being one of many compounds acting in the defence of plants suggests that alkaloids are substances formed when the organism is detoxifying poisonous substances arising from its normal metabolism. It has also been claimed that alkaloids might constitute a suitable reservoir of nitrogen for the plant (Samuelsson, 1999). Data speaking against such an hypothesis has been presented by Baldwin and Ohnmeiss (1994). They administered nicotine exogenously to *Nicotiana sylvestris* and observed that the compound was catabolised to nornicotine and myosmine. However, the plant did not recover nicotine nitrogen to invest it in other metabolic processes, even under conditions of nitrogen-limited growth, implying that nicotine is not used as a nitrogen storage form. It has also been speculated that nicotine might function as protection against detrimental effects of UV light. The data published by Baldwin and Huh (1994), and Kutchan (1995), however, do not give the hypothesis that nicotine function as a filter for UV-radiation any support.

Alkaloids may occur in every part of the plant, but usually some tissues contain higher alkaloid levels than other tissues. Bark, leaves and fruit are often rich in alkaloids. An active transport of alkaloids from one organ to another has been observed in many plants. Thus, the organ with the highest alkaloid content is not necessarily the place where the alkaloids are formed. For example, in the tobacco plant it has been shown that most of the nicotine is formed in the roots of the plant and transported to the leaves. A small amount of nicotine is also synthesised in the stem of the tobacco plant (Samuelsson, 1999). Within the plant cells, the alkaloids usually occur in the vacuoles.

5. Occurrence in food plants

There are various possibilities to be exposed to nicotine through the diet. Nicotine is a natural constituent of some food plants producing this alkaloid endogenously. Nicotine may also be a constituent of commercial products. Besides being found in products manufactured by the tobacco industry, nicotine is used in medicinal products and pesticides. For medical purposes nicotine is available in the form of chewing gum, nasal spray and nicotine-impregnated patches to be used by persons who want to stop smoking/snuffing. Insecticides with nicotine as active component are used particularly in gardening and organic farming. Residues of nicotine may, therefore, be present on the surface of food plants. A surface contamination is also possible from environmental nicotine coming from cigarette smoke of the surrounding air. In the following section, the investigators have tried to control most of these factors, aiming to determine specifically the level of nicotine endogenously produced by food plants.

5.1. Occurrence of nicotine in fresh food plants

There are only five reports on the nicotine content of Solanaceous food plants. Four of these analytical studies, based either on GC-MS or an radioimmunoassay, have a low limit of detection and has found rather similar levels of nicotine in the four investigated food plants. The fifth study (Sheen, 1988) measured the nicotine content

by a gas-liquid chromatographic technique with a comparatively high limit of detection. All the values on nicotine content of food plants reported by Sheen (1998) are significantly higher than the values reported by other investigators. This could be due to methodological differences (Siegmund et al., 1999a), but also other explanations have been suggested. For example, Siegmund et al. (1999a) criticised the study of Sheen (1988), claiming that no attempts seem to have been made to evaluate whether the products had been contaminated by nicotine from the environment. As the result of this study has been questioned, conclusions are based on data presented by the other four studies.

Nicotine has been detected in four food plants - potatoes, tomatoes, eggplant and pepper. No studies have reported failure to detect nicotine in less well-known Solanaceous food plants.

Four studies have analysed for nicotine in potatoes, *Solanum tuberosum* (Table 1). The studies of Siegmund et al. (1999b) and Domino et al. (1993) found non-peeled potatoes to contain similar low levels of nicotine, 4.5 and 7.1 μg/kg fresh weight, respectively. Two other studies did not report on the nicotine content of non-peeled potatoes, but on the nicotine content of potato peel and potato flesh of a single variety separately. One of these studies found high levels of nicotine but this was the criticised study of Sheen (1988). The two studies made different observations. Davis et al. (1991) found higher nicotine contents in potato flesh (15.3 μg/kg) than in potato peel (4.8 μg/kg), whereas Sheen (1998) found no nicotine in the flesh but extremely high levels in peel (1480 μg/kg). The author speculated that the role of nicotine in potato skin could be to strengthen the defence against pathogenic fungi

TABLE 1. Nicotine contents in tomato, potato, eggplant and green pepper.

Food Source	Castro & Monji, 1986	Sheen, 1988*	Davis et al., 1991	Domino et al., 1993	Siegmund et al., 1999
POTATO	Not included in study	Potato flesh: not detectable Potato peel: 1480 μg/kg	Potato flesh: 15.3±1.7 μg/kg (n=6) Potato peel: 4.8±0.8 μg/kg (n=6)	7.1±5.9 μg/kg (n=11)	4.5±2.2 μg/kg (n=6)
TOMATO	6.0±2.4 μg/kg (n=6)	231 μg/kg	5.1±0.8 μg/kg (n=6)	4.1±1.8 μg/kg (n=8)	2.4±1.2 μg/kg (n=7)
EGGPLANT (AUBERGINE)	100 μg/kg	265 μg/kg	Not detectable (n=6)	Not included in study	1.9±0.7 μg/kg (n=4)
GREEN PEPPER	5.7±0.0 μg/kg (n=2)	315 μg/kg	Not detectable (n=6)	Not detectable	3.7; 5.8; 6.1 (n=3)
YELLOW PEPPER	Not included in study	Not included in study	Not included in study	Not included in study	9.0 μg/kg (n=1)
RED PEPPER	Not included in study	Not included in study	Not included in study	Not included in study	5.9 μg/kg (n=1)
GREEN PEPPERONI	Not included in study	Not included in study	Not included in study	Not included in study	8.7; 6.3 (n=2)

Notes: The contents had been measured from fresh ripe fruits.
* Recalculation of ppm dry weight (d.w) to μg/kg fresh weight (f.w.) was made in the following way:
X ppm d.w. = 1000X μg/kg d.w.
X μg/kg d.w = 0.1X μg/kg f.w (assuming a water content of 90% in vegetables)

and bacteria. As indicated above, Siegmund et al. (1999b) suggested that the presence of a comparatively high level of nicotine in peel but not in the flesh could be due to surface contamination from the air, but they presented no analytical data to support their suggestion. Thus, the reason for the difference in result between the two studies is not known. It could be due to different analytical techniques being used (gas-liquid chromatography as opposed gas chromatography-mass spectrometry), different potato varieties being investigated, or different levels of environmental nicotine contamination occurring in the samples. The second of the explanations, a difference between the potato varieties, seems less likely. Siegmund et al. (1999b) studied the nicotine contents in six different potato varieties from two different harvesting seasons without finding any significant differences between variety and year.

As shown in Table 1, four of the five studies found red and ripe tomato (*Lycopersicum esculatum*) fruits to contain between 2.7 and 9.1 μg nicotine per kg fresh weight (Castro and Monji, 1986; Davies et al., 1991; Domino et al., 1993; Siegmund et al., 1999b). The highest nicotine content reported in a single fruit in these studies were 9.8 μg/kg fresh weight (Castro and Monji, 1986). There was only marginal differences in nicotine content between different varieties of tomatoes (Siegmund et al., 1999b). Davis et al. (1991) noted that fresh locally grown tomatoes (9.6 μg/kg fresh weight) contained somewhat higher levels of nicotine than tomato fruits purchased on the local market (5.1 μg/kg fresh weight). The fifth study detected much higher nicotine levels (231 μg/kg) in tomatoes using a gas-liquid chromatographic technique (Sheen, 1988).

Castro and Monji (1986) and Siegmund et al. (1999b) studied the variation in nicotine content during ripening of the tomato fruit. Both groups of investigators

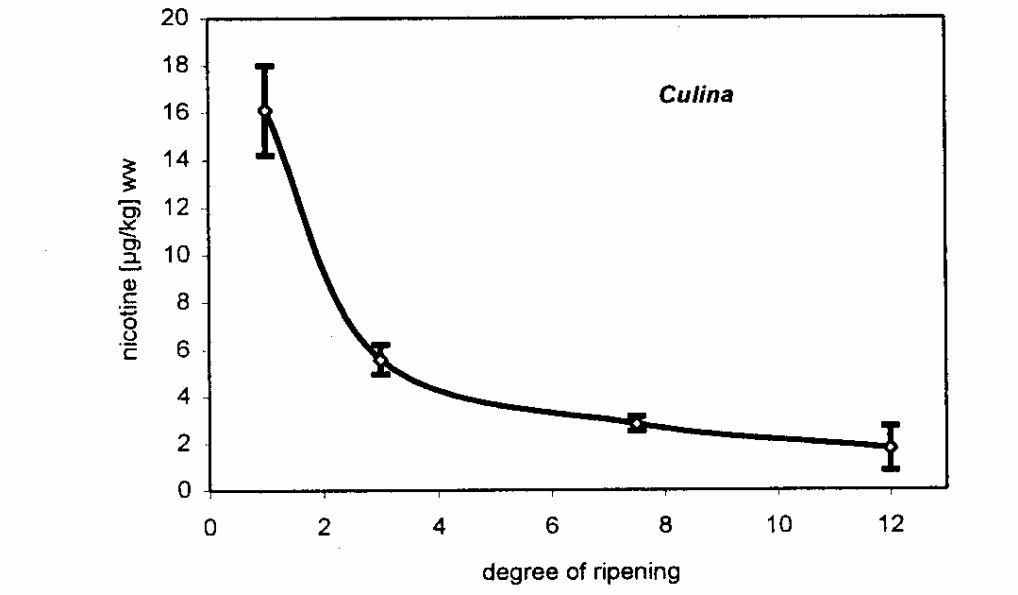

Figure 4. Nicotine levels in fruits of the tomato variety Culina at various stages of ripening (from Siegmund et al., 1999b). Reprint with permission from Siegmund et al. (1999) Journal of Agricultural and Food Chemistry 47:3113-3120. Copyright 1999 American Chemical Society.

came to the conclusion that the nicotine content is highest in unripe, green fruits and lowest in ripe fruits. This is illustrated by Figure 4, which shows a clear relationship between the level of nicotine in tomato fruits of the variety Culina and the degree of ripening (Siegmund et al., 1999b). The decrease in nicotine content during ripening occurred also in other varieties but was less pronounced. Green unripe tomatoes are in some countries used as food, for example in relishes and pickle preparations.

Different varieties of sweet pepper (*Capsicum annum*), the ordinary green, yellow or red pepper, and pepperoni, have been analysed for nicotine. Four studies analysed for nicotine in green pepper, but only one study (Siegmund et al., 1999b) looked at more than one variety. All varieties analysed by Siegmund et al. (1999b) contained detectable but low levels of nicotine - 3.7-9.0 μg/kg fresh weight (Table 1). Low levels of nicotine in green pepper (5,7 μg/kg) were observed also by Castro and Monji (1986), whereas Davis et al. (1991) were unable to detect nicotine in green pepper. Sheen (1988), on the other hand, detected as much as 315 μg/kg fresh weight.

The largest variation in nicotine content among food plants has been reported for eggplant fruits. Whereas one study was unable to detect nicotine in 6 samples of eggplant fruit (Davis et al., 1991), and Siegmund et al. (1999) only detected around 2 μg/kg fresh weight, as much as 100 μg/kg fresh weight was reported by Castro and Monji (1986).

5.2. Nicotine contents in cooked and processed food

Most studies investigating the influence of food processing on the nicotine content of food products were performed with tomatoes. Sheen (1988) reported that in contrast to fresh tomatoes, that contained 231 μg/kg nicotine, tomato ketchup and tomato paste contained levels below the detection limit (which was 100 μg/kg in their study). Sheen speculated that the absence of nicotine in processed products could be due to enzymatic oxidation of nicotine and other chemical reactions occurring during processing. As pointed out more than once, the value if these observations are questionable.

Siegmund et al. (1999b) found an average nicotine contents of 5.3 μg/kg in tomato paste, 4.5 μg/kg in tomato sauce and 7.3 μg/kg in tomato ketchup. These are low but slightly higher levels compared to the levels in fresh tomatoes (non detectable - 3.9 μg/kg f.w.). Commercial samples (n=4) of peeled tomatoes sold in cans contained non-detectable levels of nicotine up to 4.4 μg/kg f.w., whereas marketed samples (n=4) of tomato sauce had a slightly higher nicotine content (4.5-6.2 μg/kg f.w.) than fresh tomatoes. The investigators related the observed increase in nicotine contents to the decreased water content of these products. A slight increase in nicotine content of some commercial tomato products, in relation to the level in fresh fruits have been noted also by Castro and Monji (1986). One of the tomato products studied by these investigators was a type of peeled tomatoes containing as much as 52 μg/kg nicotine. Other products (a tomato paste and a tomato sauce) contained 11 and 3 μg/kg, respectively. The investigators gave no reason for the increased nicotine level in some of the studied products. Siegmund et al. (1999b), however, commented on this finding and suggested that the high nicotine level in the peeled tomatoes reported by

Castro and Monji (1986) either illustrate an exceptionally high level of nicotine in the tomato variety used for this product, or was a result of contamination of the sample with nicotine during harvesting, processing or analysis. Neither Siegmund et al. (1999), nor Castro and Monji (1986) commented on the speculative possibility that spices added during processing might have influenced the level of nicotine in the processed food product.

In the study of Siegmund et al. (1999b), all samples of cooked potatoes and French fries contained nicotine. The levels were similar in raw (4,5 μg/kg) and cooked (3,3-4,6 μg/kg) potatoes. The French fries contained slightly higher levels of nicotine (6,9 and 11,5 μg/kg) than expected, possibly because of their higher percentage of dry matter (Siegmund et al., 1999). Sheen (1988), who only detected nicotine in the peel of fresh potatoes, was unable to detect nicotine in frozen slices of potatoes.

The studies on influence of food processing reported above indicate that nicotine is thermally stable and do not evaporate during processing.

5.3. Occurrence of tobacco alkaloids other than nicotine in food plants

Since the tobacco plant in addition to nicotine is known to contain nicotine metabolites and the alkaloids nornicotine, anabasine and anatabine (Figure 3) (Petterson et al., 1991), it is natural to ask the question whether genetically related Solanaceous food plants also contain these additional tobacco alkaloids and metabolic products. However, no food plants have hitherto been studied for whether they contain nornicotine, anabasine and anatabine, or not. Davies et al. (1991) and Domino et al. (1993) have reported, however, that no cotinine (a nicotine metabolite) could be detected in potatoes, tomatoes, eggplants and green pepper.

Other Solanaceous plants than the ones commonly used as food have been shown to contain these other tobacco alkaloids, and in some cases accidental consumption of such plants have led to severe poisoning. The most notable case is the tree tobacco, *Nicotiana glauca*, which contain anabasine as the main alkaloid. Also the shrubs Day Blooming Jasmine (*Cestrum diurnum*) and Night-blooming Jessamine (*Cestrum nocturnum*) contain tobacco alkaloids. The former plant contains nicotine, nornicotine, cotinine and myosimine, whereas the latter plant contains nicotine and nornicotine (Halim et al., 1971). These are not food plants, but ingestion of a large number of fruits of *C. diurnum* has caused grave poisoning to children and animals in the Southeast of the United States. The fruits of *C. diurnum* are, however, consumed by birds without problems. In Australia, various species of the genera *Duboisia* produce nicotine and nornicotine (Griffin, 1985). One of these nicotine-producing species, *Duboisia hopwoodii*, is used by the Aborigines as chewing tobacco.

5.4. Nicotine used as pesticide

The biosynthetic pathways for nicotine in plant species other than the members of Solanaceae have not been well characterized. For example, conflicting and controversial data concerning occurrence of nicotine in tea can be found in the literature. The tea plant (*Camelia sinensis*) is not known to synthesise nicotine (Davis et al., 1991). Therefore, contamination from the use of nicotine as an insecticide has been proposed as a source of nicotine in tea (Sheen, 1988; Davis et al., 1991). Two brands of instant tea that Sheen (1999) analysed were shown to contain five to ten-

fold higher nicotine levels than fresh food products of Solanaceous plants. The investigator speculated that the unexpected finding could be explained by the fact that tea is mainly produced in developing countries where nicotine is used as an effective and cheap insecticide. Also Siegmund and co-workers (1999b) have reported surprisingly high concentrations of nicotine in all the eight samples of tea leaves analysed (163,8 - 1593 μg/kg). The investigators chose not to speculate on the origin of the nicotine.

The fact that nicotine in some countries is used as pesticide, especially in gardening, raises the question whether it can lead to contamination not only of tea but also of Solanaceous food plants. According to Idle (1989), it is hardly surprising that imported foodstuffs might contain high levels of nicotine, when nicotine is still widely used in the developing world as a cheap and effective insecticide. For example, in Zambia nicotine is used as an acaricide, fungicide, insecticide and repellent. The farmers soak tobacco leaves in water, add soap, and use the solution for spraying on plants. Nicotine solutions can also be prepared from cigarette ends as they contain most of the nicotine of the cigarettes (Malaya and Banda, 1994). Although used for quite a while as pesticide, no insects have yet developed resistance against nicotine (Kutchan, 1995).

Nicotine is one of more than 20 natural pesticides of botanical origin used in organic farming. Organic industry promotes its products by claiming that they carry lower amounts of pesticide residues. Pesticides of natural origin are, however, accepted as pesticides by organic growers without referring to them as pesticides. Residues of pesticides of natural origin are rarely quantified in official control of pesticide residues. Moore et al. (2000), however, analysed different baby foods for residues of eight organochlorine pesticides and five natural botanical pesticides, one of them being nicotine. They tested products made of applesauce, pears, winter-squash and carrots originating from two commercial producers using traditional farming, and one producer using organic farming. Residues of nicotine (level of detection was 0.66 μg/kg) were neither found in baby foods produced from conventionally grown fruits and vegetables, nor in baby foods produced from organically grown fruits and vegetables. These results support the conclusion that most nicotine detected in Solanaceous plants has been endogenously synthesised.

In Denmark, Iceland, Norway and Sweden it is not formally allowed to use nicotine-containing pesticides. Neither the National Food Administration in Sweden, nor the Danish Veterinary and Food Administration has, however, analysed for nicotine residues to control that nicotine is not used. In Finland there is at least one insecticide product with nicotine on the market (Tuomaala, personal communication).

5.5. Nicotine content in genetically modified food plants

There are two aspects of genetic modification of Solanaceous food plants, which should be mentioned in relation to tobacco alkaloids. Firstly, metabolic engineering of plants serving as commercial sources of nicotine could enhance *classic breeding* in the efforts to develop plants with optimal alkaloid patterns. Such Solanaceous plants may serve as important sources of pharmaceuticals, but they are unlikely to be traditional food plants. However, there is yet no legal guaranty that this is not the case. Secondly, the Solanaceous plants, including the Solanaceous food plants, are

particularly easy to modify with *modern biotechnology*. When genetic modification is used to improve food plants, it is required that the levels of nicotine, or the levels of other tobacco alkaloids, are not substantially increased in the genetically modified product.

Some interesting observations in relation to risk assessment of genetically modified plants have been done in transgenic *Nicotiana tabacum* and *N. rustica*. Fecker et al. (1993) established hairy root cultures of transgenic *N. tabacum* varieties carrying two direct repeats of the bacterial lysine decarboxylase (LDC) gene controlled by the cauliflower mosaic virus (CaMV) 35S promoter. The introduced gene was efficiently expressed. The activity was sufficient to increase the cadaverine levels of the cultured transgenic cells. One cell line, SR#/1-K1,2 increased the expression from around 50 μg/g dry mass (control cultures) to about 700 μg/g dry mass. Some of the overproduced cadaverine of this line was used for the formation of anabasine, as shown by a 3-fold increased level of this alkaloid. In transgenic lines with lower LDC activity the changes of cadaverine and anabasine levels were correspondingly lower and sometimes hardly distinguishable from controls. Feeding of lysine (a precursor of cadaverine) to root cultures, even to those with low LDC activity, greatly enhanced cadaverine and anabasine levels, while the amino acid had no or very little effect on controls and LDC-negative lines. Hamill et al. (1990) transformed *Nicotian rustica* with a construct containing the yeast *Saccharomyces cerevisiae* gene coding for ornithine decarboxylase, driven by the powerful CaMV 35S promoter, and an upstream duplicated enhancer sequence. The newly introduced gene was constitutively expressed during the growth cycle in root cultures. The presence of the yeast gene and enhanced ornithine decarboxylase activity was associated with an enhanced capacity of cultures to accumulate both putrescine and the putrescine-derived compound nicotine (2-fold increase). The investigators draw the conclusion that genetic manipulation can enhance the flux through pathways to plant secondary products.

In another study, the gene for *Vitreoscilla* hemoglobin (VHb) driven by the 35S CaMV promoter was introduced and expressed in *N. tabacum*. Transgenic tobacco plants expressing VHb exhibited enhanced growth, on average 80-100% more dry weight after 35 days of growth compared to wild-type controls (Holmberg et al., 1997). Furthermore, the germination time was reduced from 6-8 days for wild-type tobacco to 3-4 days, and the growth phase from germination to flowering was 3-5 days shorter for the VHb-expressing transgenes. Transgenic plants contained, on average, 30-40% more chlorophyll and 34% more nicotine than controls. VHb expression also resulted in an altered distribution of secondary metabolites: in the transgenic tobacco plants anabasine content was decreased 80% relative to control plants.

All three studies show that it is possible to influence the tobacco alkaloid levels in Solanaceous plants by genetic modification. They also show that it might be advisable to control for this possibility in the risk assessment of Solanaceous food plants, not at least when the newly introduced genes may influence the availability of precursors for tobacco alkaloid synthesis.

6. Dietary exposure to nicotine

To calculate the dietary exposure to nicotine from the Solanaceous food plants potato, tomato, eggplant and green pepper, data are needed on the levels of nicotine and on the daily total intake of these food items from all sources (Table 2). For tomatoes, for example, this requires information on consumption of fresh tomato fruit, ketchup, tomato sauce, etc., as well as the amounts of these food items in ready to eat products such as pizzas and canned food. Such data, if available, are obtained from various sources. Information on the average daily per capita intake of potatoes from all sources during the period 1993-1999, 168.6 gram, was obtained from Statistics Sweden (2000), as were the information on average tomato intake, 21.6 gram/day.

Because no satisfactory information on the consumption of eggplant was available, import/export statistics for year 2000 (Swedish Board of Agriculture, personal communication) were used to calculate the daily per capita intake, assuming that the entire eggplant fruit is used in food preparation and that there is no wastage. Also the consumption of sweet pepper is poorly investigated. The data used in our calculation was obtained from Riksmaten 1997/1998, a database managed by the National Food Administration, which, however, only gave information on consumption of green pepper.

Based on statistical information, the Danish daily per capita intake of potato in 2001 was calculated to be 156 g (Statistisk årbog, 2002), of tomato in 2000 62 g (Grønsager på Friland, 2001), and of sweet pepper in 1996-1997 5.5 g (Grønsager på Friland, 1998). There is no statistical information available on eggplant consumption in Denmark. However an average intake of 2.7 g/capita/day was calculated from national household interviews (Fagt, Danish Veterinary and Food Administration, personal communication).

The average nicotine content of potatoes, tomatoes, eggplant and sweet pepper (green) was calculated from the data presented in Table 1 (Castro and Monji, 1986; Davis et al., 1991; Domino et al., 1993; Siegmund et al., 1999), neglecting the deviating data of Sheen (1988) in the calculation. It is clear that potatoes give the highest contribution to our dietary nicotine exposure, on average around 0.9 and 1 μg/day in Denmark and Sweden, respectively (Table 2).

TABLE 2. Average daily consumption, average nicotine contents and average daily dietary nicotine exposure from tomato, potato, eggplant and sweet pepper.

Food Source		Average daily consumption (g/day)	Average nicotine content (μg/kg)	Average daily dietary nicotine exposure (ng/day)
TOMATO	- Sweden	21.6	4.4	95.0
	- Denmark	62		273
POTATO	- Sweden	168.6	5.8	977.9
	- Denmark	156		905
EGGPLANT	- Sweden	0.71¤	34.4	24.4
	- Denmark	2.7		93
SWEET PEPPER	- Sweden	0.27*	5.4	1.5
	- Denmark	5.5**		30

¤ Swedish import of eggplant in 2000 (2 297 000 kg) divided with number of citizens in 2000 (8872294) and 365 days; * only green pepper; ** green, yellow or red pepper

Less is derived from tomatoes (95 and 273 ng/day in Sweden and Denmark, respectively), eggplant (24 and 93 ng/day) and sweet pepper (2 and 30 ng/day). It should be stressed that the calculated nicotine exposure is based on the *average* consumption of vegetables. Dietary habits are individual and, in particular, vegetarians are expected to consume significantly more vegetables per day than non-vegetarians. They are therefore expected to ingest larger doses of nicotine than non-vegetarians. But the exposure is still very low. There are also some differences in vegetable consumption between men and women. Swedish women consume more vegetables than men do but men consume more potatoes than women. (Becker, 1999). It is also likely that there are some differences in consumption by geographical region and level of education. The differences will not have any main influence on the conclusion that the dietary nicotine exposure is low. The average daily total dietary nicotine intake of 1.1 μg in Sweden and 1.3 μg in Denmark could be compared with the nicotine amount assumed to be absorbed from a single cigarette, 900 - 1 700 μg.

7. Toxicokinetics

Nicotine is rapidly absorbed from the lungs, but poorly from the mouth and nasopharynx (Rang et al., 1999). Once nicotine has entered the systemic circulation, the toxicokinetics of nicotine is expected to be similar irrespective of the route it entered. Figure 5 illustrates how the plasma concentration of nicotine varies after smoking a cigarette, chewing a nicotine gum and taking nicotine intranasally (Russel et al., 1983).

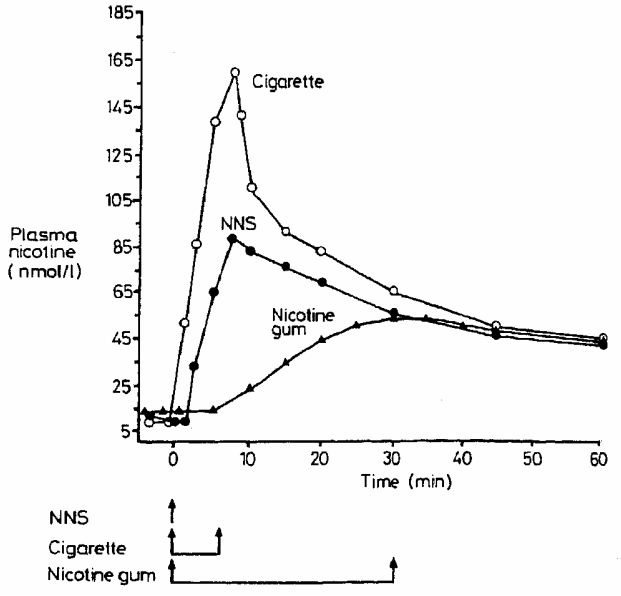

Figure 5. Mean plasma nicotine concentrations in 3 subjects after smoking a cigarette, chewing 2-mg Nicorette® nicotine gum, and taking 2 mg nicotine intranasally. (Conversion: 1nmol/L ≈ 0,16/ml). Reprint with the permission of the British Medical Journal Publishing Group (Russel et al., 286 (1983):683-684).

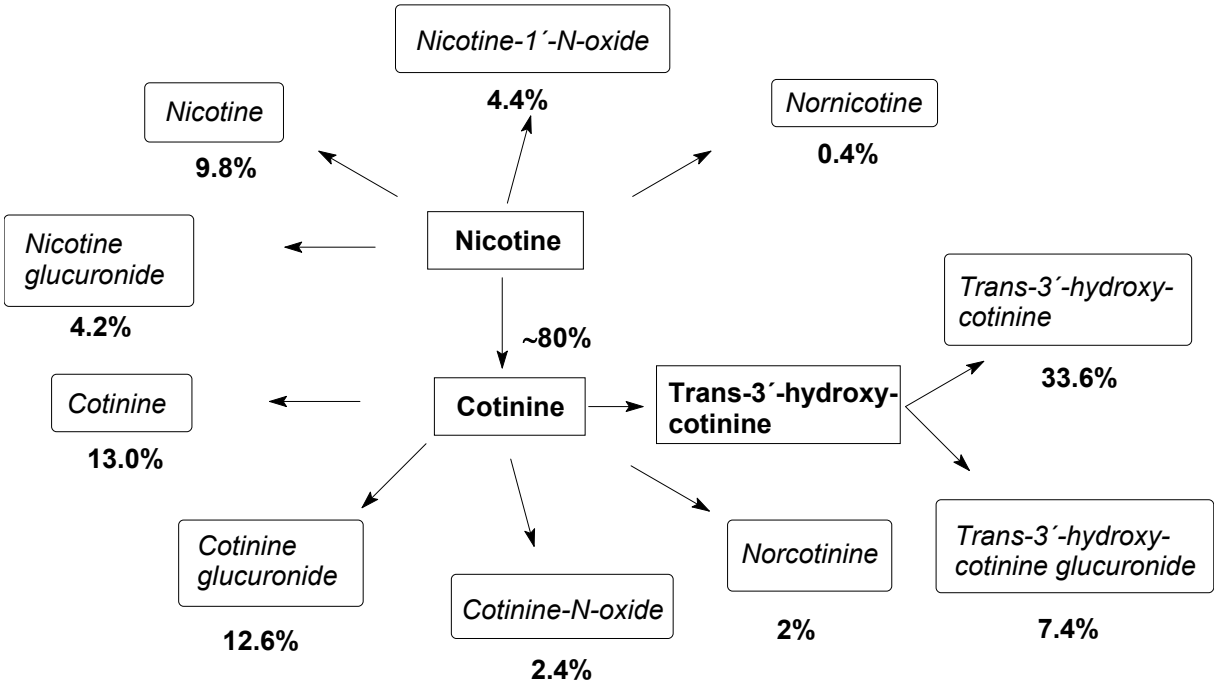

Figure 6. Quantitative scheme of nicotine metabolism. Compounds in italics have been detected excreted in urine. Numbers associated indicate percentage of systemic dose of nicotine (after Benowitz et al., 1994).

When nicotine is taken into the body through the lungs, it is absorbed via the lung alveoli. All, or nearly all nicotine, will be absorbed into the systemic circulation and

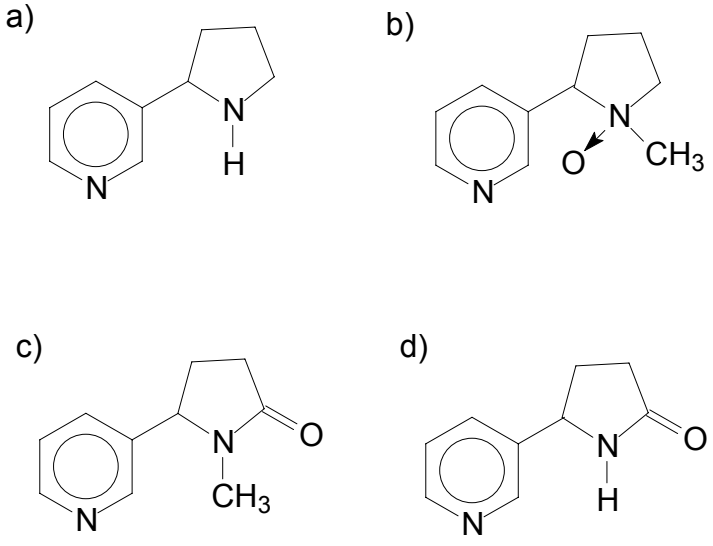

Figure 7. Chemical structure of the nicotine metabolites: a) nornicotine; b) nicotine-1-oxide; c) cotinine; and d) norcotinine.

reach body organs, including the liver and kidneys. The liver converts nicotine to a large number of metabolites (Figure 6), the main one being produced by a hydroxylation attack leading to the opening of the pyrrolidine ring and reclosing of the ring to form cotinine (approximately 80% of the metabolites). As shown in Figure 6, a number of different cotinine- and nicotine-derivatives are excreted in urine (Gorrod, 1993; Benowitz, 1996). A small percentage, usually 5-10 percent, is excreted unchanged as nicotine (Benowitz, 1996). It should be stressed, however, that there is an inter-individual variation in nicotine metabolism. The chemical structure of some of the metabolites is shown in Figure 7.

Uptake of nicotine after oral administration of the compound can not be directly compared with the uptake after pulmonary exposure to the compound. Oral exposure to nicotine leads to molecules reaching the gastrointestinal tract, and being absorbed through the gut wall into its blood veins. The nicotine molecules are transported in the gut wall blood veins to vena porta, which directs the blood to the liver, before it is allowed to reach the systemic circulation. Thus, nicotine will to some extent be metabolised by the bacteria and enzymes in the gastrointestinal tract, and to a large extent by the hepatic enzymes, before the molecules are distributed in the body. It has been calculated that 60-70 percent of orally supplied nicotine is metabolised by this type of first-pass metabolism before ever reaching the systemic circulation (Benowitz et al., 1991). It should be stressed, however, that there are big interindividual differences in metabolising rates of nicotine (Benowitz et al., 1982).

The half-life of nicotine in man averages 2-3 hours and that of cotinine about 17 hours (Benowitz, 1996). The longer half-life of cotinine is one of the main reasons why cotinine is used as a biomarker of exposure to tobacco smoke. Cotinine has been described as a specific and sensitive marker of exposure of tobacco smoke and a useful and reliable indicator of nicotine intake (Idle, 1989).

The identification of nicotine as a natural constituent of food plants, has led some investigators to question the relevance of cotinine as a biomarker of exposure to tobacco smoke (Idle, 1989; Davis et al., 1991; Domino et al., 1993; Benowitz, 1996; Siegmund et al., 2001). It has been suggested that nicotine in food might falsely indicate environmental tobacco smoke exposure ("passive smoking"). To place exposure to nicotine from food in perspective, one needs to compare the average intake of nicotine from food with that from environmental tobacco smoke (Benowitz, 1996). Figure 8 shows the distribution of serum cotinine levels in a large US population between 1988 and 1991 (Pirkle et al., 1996). The relationship between the nicotine intake in smokers and blood cotinine can be expressed mathematically. The average conversion factor (*K*) converting the blood level of cotinine to daily intake of nicotine in adult smokers has been estimated to 0.08 mg/24h/ml (range 0.05-0.10; coefficient of variation, 21.9 percent) (Benner et al., 1989). Thus, a cotinine level of 300 ng/ml (a typical value for a smoker) corresponds to a daily intake of 24 mg nicotine. To ingest this amount of nicotine from potatoes or tomatoes would require a consumption corresponding to either 3.4-5.3 tons potatoes, or 2.5-8.9 tons tomatoes.

Since clearance of nicotine is similar for smokers and non-smokers, the *K* factor is expected to be similar in non-smokers and smokers (Benowitz, 1996), and can be used to calculate the nicotine exposure in passive smoking. A plasma level of 1

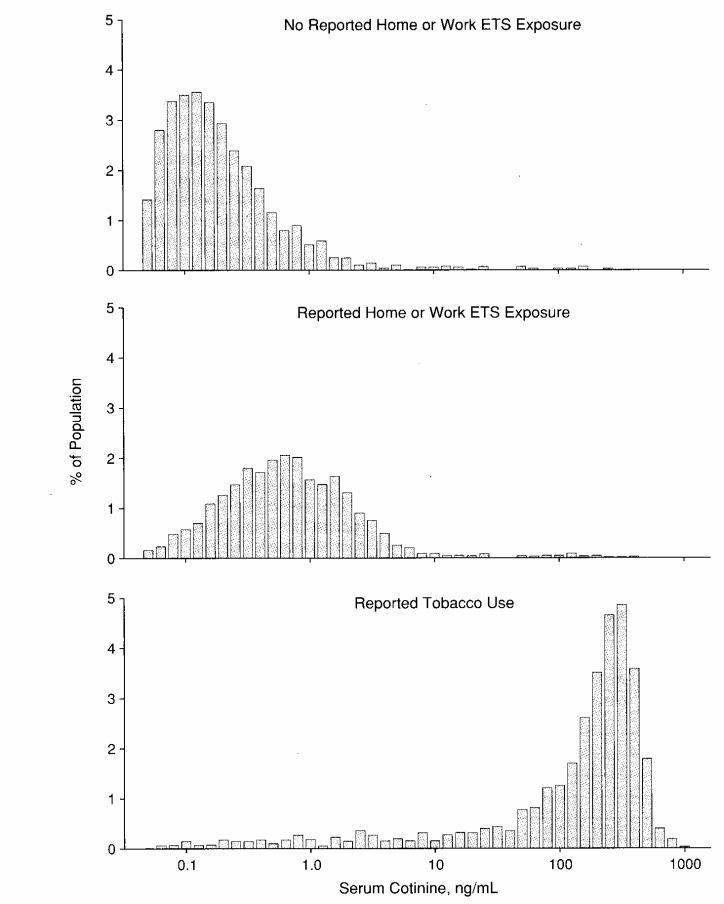

Figure 8. Distribution of cotinine levels in the US population aged 4 years and older by reported environmental tobacco smoke (ETS) exposure and tobacco use. Data from the Third National Health and Nutrition Examination Survey, 1988-1991. Reprint with the permission of the publisher and the authors. Reprint from Pirkle et al. in JAMA 275(1996):1233-1240.

ng/ml cotinine is typical for persons exposed to environmental tobacco smoke at home or in the working environment ("passive smoking"). This would correspond to an exposure of 0.08 mg (= 80 000 ng) nicotine per day. Thus, exposure to dietary nicotine is negligible compared to nicotine exposure from passive smoking. Even if we leave out of account the fact that less than half of the dietary nicotine is absorbed by the body (due the first pass effect), one still have to consume enormous quantities of vegetables to be exposed to 80 000 ng nicotine. This amount of nicotine would correspond to either 11-18 kg potatoes or 8-30 kg tomatoes. Therefore, the dietary exposure to nicotine from Solanaceous food plants *per se* is not considered to be of any health concern.

8. Toxicity and poisoning

The risk for nicotine poisoning is much higher when working with the compound than in ordinary smoking and when consuming food plants containing the compound. Although being present in low to moderate concentration in tobacco products, nicotine is concentrated into insecticides that are used in agriculture or horticulture.

Exposure to nicotine occurs to a variety of people working with the tobacco plant or handling nicotine insecticides both by the dermal and ingestion routes.

Field workers harvesting tobacco leaves often develops "green tobacco sickness", especially if they harvest wet, uncured leaves. Dermal absorption from the fluid on the leaves is the most important exposure route, but inhalation of aerosols may also occur. Symptoms take several hours to manifest themselves and include headache, nausea, dizziness, abdominal cramps and vomiting. Recovery usually takes place within one day. Smoking tobacco affords some protection, most likely because a tolerance to nicotine develops (Gehlbach et al., 1974).

When nicotine is concentrated into an insecticide, accidental dermal exposure becomes a significant concern which can warrant hospitalisation (Faulkner, 1933). Due to the extreme toxicity of nicotine and lack of a specific antidote, ingestion of nicotine insecticides can be fatal (Smith, 1951). Nicotine is especially toxic to children (Singer and Janz, 1990). Poisoning usually occur because of improper storage in a beverage container or because of suicide (Hearn, 1973). However, nicotine is rarely used for suicide or to poison or kill a victim because of its strong odour and taste (Gindhart, 1939).

A part of the risk profile with nicotine is the potential to influence the activity of enzymes participating in metabolism of potentially carcinogenic agents. Iba et al. (1999) recently examined the dose-cytochrome P-450 (CYP) 1A1/2 induction response relationship and the tissue specificity of the induction by orally administered nicotine (20, 60 or 200 mg/kg diet) in the rat. Nicotine induced CYP1A1 in the lung and kidney in a dose-dependent manner and in the liver at the high nicotine dose only, whereas CYP1A2 was induced in the liver dose-dependently and in the kidney at the high nicotine does only. An up-regulation of mRNA levels was observed in all three tissues, particularly at the high dose. The plasma nicotine levels at which CYP1A induction was maximal were comparable to those reported in smokers, suggesting that nicotine may induce CYP1A1 in human smokers. The level of nicotine in food is to low to produce such an effect.

Nicotine also has many pharmacological effects. At low doses, such as those obtained after inhalation of cigarette smoke, nicotine causes hypertension, stimulates respiration and secretion from several glands. An ordinary cigarette contains about 0.8 g of tobacco, corresponding to 9-17 mg nicotine. Smokers usually absorb about 10% of the nicotine available, but this fraction varies greatly with type of cigarette and the habit of the smoker (Rang et al., 1999). The action of nicotine may differ between organs and be in opposing directions - the net effect depends on interactions. Nicotine has an unusual dose-response relationship for many pharmacological effects; low doses produce stimulation and higher doses relaxation or sedation (Benowitz, 1988). Also typical for nicotine is that it, like caffeine, gives rise to tolerance and dependence (Rang et al., 1999). The most important actions of nicotine in man are summarised in Table 3. In general the biological responses tabulated are consistent with nicotine activating the sympathetic nervous system.

CNS effects attributed to nicotine include reinforcing effects, mood elevation, arousal, locomotor stimulant effects, and learning and memory enhancement. The

TABLE 3. Actions of nicotine in man (after Benowitz, 1988).

Cardiovascular	Metabolic	Central Nervous System	Endocrine
Increased heart rate Cardiac contractility Blood pressure Cutaneus vasoconstriction-Decreased Skin temperature Catecholamine release	Increased free fatty acids Glycerol Lactate	Arousal or relaxation	Increased growth hormone ACTH/ cortisol Vasopressin β-endorphin
		EEG changes	
		Tremor	Inhibition of prostacyclin synthesis

reinforcing and locomotor stimulant effects of nicotine have been suggested to be the result of an activation of the CNS dopaminergic systems, and nicotine-induced modulation of dopaminergic neurotransmission has been studied in detail. The studies of Crooks and Dwoskin (1997) indicate that the CNS effects resulting from nicotine exposure may not be due solely to nicotine, but may result, at least in part, from actions of nicotine metabolites. Support for this conclusion has been presented also by Bardo et al. (1999), Green et al. (2001), and Dwoskin et al. (2001).

Nicotine is very toxic at higher doses. At the cellular level, nicotine interacts with nicotine acetylcholine receptors, thereby opening cat ion channels and causing neuronal excitation. Nicotine also effects the central nervous system. The central effects are complex and cannot be summed up overall simply in terms of stimulation or inhibition (Rang et al., 1999). Symptoms as nausea, vomiting, pallor, weakness, dizziness, headache, sweating and abdominal pain can be expected from excessive doses of nicotine (Benowitz, 1988). Typically individuals become intolerant to tobacco (Gindhart, 1939). The most characteristic sign of nicotine poisoning is severe prostration with gastric upset, and profound cardiovascular collapse. The most frequent late symptoms observed is pre-cordial stress with dyspnea varying in severity. At the same time there are no clinical evidence of heart disease. The acute lethal dose in man is 50-100 mg, which approximately corresponds to the nicotine content of 5 cigarettes. However, when smoking most of the nicotine in a cigarette is destroyed by the heat or spread into the air. Death occurs as a result of respiratory arrest (Samuelsson, 1999).

The toxicity of the tobacco alkaloids have been compared in bioassays. Such studies have revealed natural l-isomers to be more toxic than d-isomers. Exchanging the methyl group on the nitrogen of the saturated ring in nicotine with a hydrogen atom produces a more active compound. This difference is particularly striking when the saturated heterocycle is a six-membered piperidine - anabasine (Figure 3) being ten times as active as nicotine in an assay using the plant-louse *Aphis rumicis* (Soloway, 1976).

8.1. Anabasine poisoning

Poisoning from other tobacco alkaloids than nicotine has been reported, but not in connection with traditional food. Up to 1969 seven cases of anabasine poisoning had

been published in the Soviet literature. The intoxications were usually connected with the use of anabasine to control various plant, berry and vegetable crop pests, or the malaria mosquito. The last of these intoxicated cases was a 29-year-old man arriving at the clinic ½ h after using a therapeutic clysma of "tar" for his condition of rectal prolapse. He experienced sharp general weakness, vertigo and nausea. Although the condition progressed into an unconscious and very critical state, he recovered after receiving an oxygen-air mixture and supplemental treatment (Danilin and Shabaeva, 1969). Anabasine was demonstrated in the urine and in the flask containing the liquid for the patient's clysma.

Anabasine is the main tobacco alkaloid in the tree tobacco, *Nicotiana glauca.* The fruits and leaves were found to be the richest organs in anabasine content (1.2% and 1.1%, respectively) followed by roots, flowers and stems (Khafagy and Metwally, 1968). Fatal cases has been reported after poisoning with the plant in the United States, South Africa, Australia and Thailand (Castorena et al., 1987; Sims et al., 1999; Mizrachi et al., 2000; Steenkamp et al., 2002). The Australian case was a person killed by drinking a water extract of the plant (level of anabasine in blood 2.2 mg/l). The Thai case was a person deceased after consumption of cooked leaves of the plant (anabasine detected in food extracts, and blood and urine of the diseased) mistaken them as a Thai spice. Also the South African case was an accident, where an elderly lady and her son died after consuming a supper with porridge and marog. In this case juvenile *N. glauca* was mistakenly used instead of *Amaranthus hybridus* (marog when used as food) (Steenkamp et al., 2002). Mellick et al. (1999) reported on two patients that presented at emergency with life-threatening motor paresis after having ingested *Nicotiana glauca* leaves. Severe muscle weakness, bulbar palsies, flexor muscle spasm, hypertension, nausea, vomiting, and respiratory compromise were reported.

The pronounced toxicity of the tree tobacco (*Nicotiana glauca*) has been confirmed in feeding studies with pregnant cows, sows, ewes, and goats. In addition to a number of toxic symptoms on the pregnant animals, teratogenic effects were produced in the offspring (Keeler, 1979; Keeler et al., 1981; Keller and Crowe, 1983, 1984; Panter et al., 2000). The principal alkaloid of *N. glauca*, anabasine, was isolated in large quantity and good purity and the teratogenicity of the compound tested in pigs (Keeler et al., 1984). Addition of anabasine to the pig feed resulted in teratogenic effects in the offspring.

Gardener et al. (1999) compared the toxic activity of the tobacco tree alkaloids anabaseine, anabasine and N-methyl anabasine in a mouse bioassays *in vitro* and found the alkaloids to be toxic at the doses 1.1, 1.6 and 12.4 mg/kg body weight, respectively.

9. Conclusions and recommendations

It seems reasonably well established that nicotine occurs naturally in potato, tomato, sweet pepper and eggplant but at very low levels. In the first three of these food plants the level is below 10 μg/kg, whereas fresh eggplant fruit contains up to 100 μg/kg. The chemical analysis of nicotine at these low levels requires rigorous control

of factors that might influence the analytical level. No other tobacco alkaloids have been detected in these food plants.

The average dietary exposure to nicotine from the food plants mentioned above was calculated to be 1.1 μg/day (88% from potatoes) in Sweden and 1.3 μg/day (70% from potatoes) in Denmark. This is about two orders of magnitude lower than the exposure from passive smoking and around three orders of magnitude lower than the direct exposure during cigarette smoking (around 900 - 1 700 μg nicotine is assumed to be absorbed from a single cigarette). In addition to the difference in exposure level, absorption is much lower when exposure occurs in the diet than when by the inhalation route. Furthermore, more nicotine is metabolised before systemic distribution when absorbed from the stomach.

Nicotine is very toxic at high doses. The acute lethal dose in man is 50-100 mg. At lower doses it has many pharmacological effects. In comparison, the total dietary exposure to nicotine is very low, and seems to be insignificant in relation to exposure giving toxic and pharmacological effects.

In some countries, particularly in developing countries, nicotine is used as a cheap and effective insecticide. The farmers soak tobacco leaves (or cigarette ends) in water, add soap, and use the solution for spraying on plants. Nicotine is also one of more than 20 natural pesticides of botanical origin used in organic farming. In Denmark, Iceland, Norway and Sweden it is not formally allowed to use nicotine-containing pesticides. In Finland, however, there is at least one insecticide product with nicotine on the market. As the Nordic authorities never have analysed for nicotine residues in food, it could be recommended that studies should be carried out to confirm that nicotine is not used as a pesticide, or, alternatively, measure the levels in the food plants. These recommendations of course hold true for all fruits and vegetables, not only for Solanaceous food plants.

As some well known Solanaceous food plants naturally contain nicotine, it is recommended to control that the level of this alkaloid and possibly related alkaloids are not significantly increased when plant breeders in the future develop new improved varieties of these food plants using modern biotechnology.

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