Determination of the Nicotine Content of Various Edible Nightshades (Solanaceae) and Their Products and Estimation of the Associated Dietary Nicotine Intake

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This investigation was initiated as a result of proposals in the literature that dietary nicotine intake could contribute to the level of nicotine metabolites in biological fluids such as salivary cotinine concentration. Nicotine concentration was determined in several frequently consumed vegetables from the nightshade family (Solanaceae) (i.e., tomatoes, potatoes, aubergines, and peppers), as well as in some of their processed products. The edible Solanaceae fruit analyzed in this investigation were found to contain relatively consistent amounts of nicotine in the range of 2−7 μg/kg for fresh fruits. Nevertheless, the nicotine concentrations of the investigated tomato varieties decreased significantly with increasing degree of ripening of the fruits. In addition, a variety of black as well as green teas was investigated for the nicotine content. Nicotine content in tea leaves was found to be highly variable and sometimes much larger than in the Solanaceae fruits. On the basis of the observed concentrations and the respective food consumption data for different countries, a distributive analysis of the results suggests that the mean daily dietary nicotine intake for the population of the countries for which consumption data were available is approximately 1.4 μg/day, 2.25 μg/day at the 95th percentile.

Keywords: *Nicotine; Solanaceae; dietary nicotine intake; vegetables; tea*

INTRODUCTION

The concentration of biomarkers such as nicotine metabolites resulting from exposure to environmental tobacco smoke (ETS) is expected to decrease with reduced exposure. Nicotine is known to be present in a number of vegetables. One may ask whether at some point in reduced ETS exposure could the dietary contribution of nicotine intake become significant in comparison to that from ETS exposure.

It has been reported that nicotine has a wide distribution in flora. Leete (1983) reported its presence in 12 families and 24 genera, including the nightshade family (Solanaceae). Some common vegetables belong to the biological family of nightshades, and throughout the world these plants are consumed in significant amounts. They include such basic foods as potatoes, tomatoes, and aubergines (eggplants). The function of nicotine in these plants is still not clearly understood. However, it is assumed that nicotine serves as a natural defense against fungi, bacteria, insects, and animals (Kuhn, 1964; Ames, 1983; Davis et al., 1991). To a limited extent, nicotine is used as an insecticide in some parts of the world and could be a food contaminant (Idle, 1990; Domino et al., 1993).

Only a few citations can be found in the literature that address nicotine concentration in diverse foods and the ensuing dietary intake of nicotine. Although no systematic investigations have been conducted, there is controversy on the subject. In some papers it is suggested that the contribution of dietary nicotine intake is significant when compared with exposure to ETS or by active smoking of small numbers of cigarettes (Castro and Monji, 1986; Idle, 1990; Davis et al., 1991; Domino et al., 1993). Others consider the dietary intake to be negligible unless inordinately large amounts of specific vegetables are consumed (Repace, 1994; Jarvis, 1994). Nevertheless, a confounding effect is discussed for people consuming high amounts of vegetables, in particular for vegans or vegetarians (Idle, 1990). At issue is also the suggestion that dietary habits differ between smokers and nonsmokers. A recently published study (Osler, 1998) proposed that nonsmokers have healthier diets than smokers, including a higher consumption of raw and cooked vegetables as well as higher consumption of tea.

The objective of the present study was to determine the feasibility that consumption of foods known to contain nicotine could contribute significantly to the nicotine intake. To make this judgment, the level of daily dietary nicotine intake must be estimated. In the course of this study, nicotine was determined in several fresh fruits (with emphasis on tomatoes, potatoes, aubergines, and peppers) as well as processed foods originating from these fruits (e.g., tomato paste, ketchup, French fries, etc.). Various amounts of nicotine in fresh vegetables have been reported previously (Castro and Monji, 1986; Sheen, 1988; Davis et al., 1991; Domino et al., 1993). The reports generally fail to use validated analytical methods, to consider potential contamination sources, or to perform adequate replicate determinations. On the basis of early investigations for nicotine concentrations in tomatoes of different ripening stages (Castro and Monji, 1986), a detailed examination of different varieties of tomatoes at various stages of ripening was conducted. Only limited data can be found in the literature about processed foods made from the Solanaceae vegetables (Castro and Monji, 1986; Sheen,

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1988). Therefore, commercially available products from tomatoes and potatoes were included in this investigation.

Conflicting and controversial data concerning nicotine concentrations in tea can be found in the literature, although nicotine biosynthesis in teas has not been demonstrated (Davis et al., 1991). 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). Moderately high nicotine concentrations in teas have been reported by Sheen (1988) and Davis et al. (1991). In contrast, Domino et al. (1993) did not detect any nicotine in black tea. Consequently, the role of nicotine intake via tea consumption is controversial as well (Idle, 1990; Chappell and Gratt, 1996). To address this issue, a selection of teas was included in this study. As the consumption of green tea is expected to increase due to its high antioxidative potential (Zeyuan et al., 1998), black tea as well as green tea were investigated equally.

Combining the nicotine concentrations found in the investigated foods with published values for daily dietary intake of these foods, estimates of daily dietary nicotine intake were made. Using the mean values and precision estimates, a Monte Carlo simulation was conducted to show a distribution probability of daily dietary nicotine intake.

MATERIALS AND METHODS

Materials. *Fresh Tomatoes*. Seven types of tomatoes at various stages of ripeness were obtained from an Austrian vegetable breeding station. The tomatoes were grown in greenhouses. Sampling was performed according to the degree of ripeness of the different varieties. The degree of ripening (DR) was determined using a classification system from the Sprenger Institute, Wageningen, Netherlands, that classifies the tomatoes into 12 ripening stages according to their color. DR 1 describes the unripe, green fruit, whereas DR 12 describes the fully ripe stage. To guarantee homogeneity within one set of samples, the colors of the tomatoes were determined according to Biesalski (1957). Samples were taken by nonsmokers to avoid contamination. The following varieties were chosen: *Culina, Mercedes, Furore* (round types, very common), *Favorita* (common cherry tomato), *Alteza* (common aubergine-like tomato), *Hypeel 108* (aubergine-like tomato, American breeding), and *Marinda* (beef-steak tomato). From each variety at least four samples of different ripening stages were investigated.

Potatoes. For the analysis of potatoes, six different varieties of two different harvesting years were purchased in local supermarkets.

Aubergines. Four different varieties were analyzed for their nicotine content. Two samples were purchased in a local supermarket. Two types are not commonly grown in Austria (*Pintung Long*, Chinese breeding; *Ichiban*, Turkish breeding) and were obtained from the breeding station.

Green Peppers and Pepperonis. Seven different types of peppers and pepperonis were analyzed, obtained both from a local supermarket and from the breeding station. *Bendigo* (light green pepper), *Multi* (dark green pepper), *Flamingo* (yellow pepper), and two types of pepperoni (one hot and dark green variety as well as one mild and light green variety) were received from the breeding station; two peppers (one dark green and one red) were purchased from a local supermarket.

Processed Products. Processed products mainly from tomatoes (i.e., ketchup, tomato sauce, canned tomatoes, tomato pulp, commercially available homogenized tomatoes) as well as from potatoes (i.e., French fries) were purchased from local supermarkets. The entire contents of the packages were homogenized, and aliquots were used for the analyses. For the preparation of cooked potatoes, the entire potatoes were cooked under atmospheric pressure and peeled before the analysis.

Tea. Eight commercially available types of tea (four black and four green) were analyzed. The black teas were purchased from a local supermarket, whereas the green teas were bought in a local tea shop. Nicotine was determined from tea leaves as well as from brewed tea, which was prepared by brewing tea leaves (about 3 g; corresponding to a heaped tea spoon) with boiling double-distilled water (250 mL; corresponding to a large cup). The tea was drawn for 5 min.

Possible Sources of Contamination. Special emphasis was put on possible contamination sources of nicotine. Nicotine air contamination as well as nicotine derived from chemicals or any equipment used was investigated (Siegmund et al., 1999). Smoking was not permitted in the laboratory area, and airborne nicotine levels in the laboratory were checked to ensure minimal potential for contamination. The results showed that airborne nicotine ($\leq 0.35\ \mu g/m^3$ within the laboratory, $\leq 0.60\ \mu g/m^3$ in adjoining hallways) did not represent a significant source of contamination. Another concern was the possible surface contamination of the fruit samples, especially of those samples purchased in supermarkets for which nothing is known about the history of the sample. Nicotine from fruit surfaces was found to be 2−4 orders of magnitude lower than the nicotine concentrations of the whole fruit and was of no relevance.

Sample Preparation. The fresh fruits were washed thoroughly with hot water and double-distilled water, dried, and transferred into a glass vessel of a BÜCHI homogenizer (BÜCHI Mixer B400, Büchi Labortechnik AG, Flawil, Switzerland). The homogenized samples were stored in the deep freezer at −18 °C in polyethylene boxes which were carefully cleaned with detergent, tap water, double-distilled water, and acetone. For the commercially available products, the contents of the whole package were homogenized and stored in polyethylene boxes until use.

Extraction of the Analytes. *Extraction of Fresh Fruits*. For the extraction of nicotine from plant material, a simple liquid−liquid extraction procedure was developed taking advantage of the polar character of the analyte (Siegmund et al., 1999). The homogenized samples (3−5 g) were weighed into a 10 mL screw-cap vial (Wheaton, Millville, NJ). The samples were spiked with the internal standard (82 ng of nicotine-methyl-d_3) and shaken vigorously to distribute the internal standard homogeneously within the sample. NH_4OH (1 mL) was added to achieve pH 12 (±0.5); the pH was checked regularly. Toluene (3 mL) was added. The samples were mounted onto an automatic shaking device in horizontal position to provide optimal shaking conditions. The samples were shaken overnight (∼15 h, 250 rpm). Then the samples were centrifuged (15 min, 4200 rpm) to obtain good phase separation. For samples that did not show good phase separation, a spatula tip of NaCl was added, shaken intensely, and centrifuged again. The organic extract (2 mL) was separated and reduced gently to 0.5 mL using a rotary evaporator (Zymark Turbo Vap 500, Zymark Hopkinton, MA). This final extract was injected directly into the GC-MS instrument. Cotinine, an important nicotine metabolite, could not be extracted with this method.

Cleanup Procedure. For processed products as well as for unripe tomatoes, further cleanup steps were necessary to eliminate interference from flavorings or other additional ingredients. The organic layer that was obtained after the extraction and centrifugation was not reduced to 0.5 mL but was further processed. An aliquot of 2 mL of the organic extract was separated and transferred into a new 10 mL screw-cap vial. HCl (1 mL, 0.05 N) was added and shaken on the Vortex (Ika Labortechnik AG, Janke & Kunkel, Staufen, Germany) for 1 min. If necessary to achieve good phase separation, the sample was centrifuged briefly. An aliquot of the aqueous sample was separated (0.7−0.8 mL) and transferred into a third screw-cap vial. NaOH (3 drops, 5 N) and toluene (0.5 mL) were added. The samples were shaken again for 1 min using the Vortex and centrifuged shortly. The organic

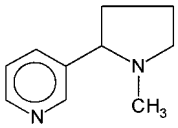

Figure 1. Chemical structure of nicotine.

layer was used directly for GC-MS measurements. The volume corrections were considered in the final calculations.

Dry Weight. The dry weight of the homogenized fruits (3−5 g) was determined in duplicate with an infrared drying system (Mettler Toledo, LJ 16 Moisture Analyzer) containing an integrated analytical balance. The drying process was performed at 105 °C until mass constancy was reached, and the dry weight was measured gravimetrically.

Gas Chromatography−Mass Spectrometry. The qualitative and quantitative determination of nicotine was performed by GC-MS (Siegmund et al., 1999). Gas chromatography was performed using a Hewlett-Packard HP 5890 II+ equipped with a mass-selective detector (HP MSD 5972). The capillary column employed was a HP 5 MS (30 m, 0.25 mm, 0.25 μm). Helium (99.999%) was used as the carrier gas. The operating conditions were as follows: a split/splitless injector was used in the splitless mode with an injection volume of 2 μL; the injector temperature was 235 °C. The following pressure program using a pressure pulse at the beginning of the chromatographic run was used: 150 kPa of helium was held for 0.5 min, then the pressure was decreased instantly at a rate of 500 kPa/min to 50 kPa; constant flow was used for the remaining time (0.87 mL/min or 24.1 cm/s, respectively). The following temperature program was used: 70 °C was kept for 1 min; then a temperature ramp of 25 °C/min was carried out to a final temperature of 280 °C, which was held for 3 min. The detector temperature was 280 °C. Electron impact ionization (70 eV) mass spectrometry was performed. The data were acquired in the selected ion mode. The following characteristic ions were used for the selective detection of the compounds: nicotine: *m/z* 84, 133, 162; nicotine-methyl-d_3 (internal standard): *m/z* 87, 136, 165.

Quantification of the Analytes. Quantification of nicotine was performed using nicotine-methyl-d_3 as an internal standard. Calibration curves were prepared using 1 mL of ethyl acetate/triethylamine (0.01%) containing variable amounts of nicotine (0, 12, 60, 120, 240 ng) and a constant concentration of the internal standard nicotine-methyl-d_3 (82 ng). Linear regression was used for all calibrations. The suitability of the regression models was checked by appropriate statistical methods (Funk et al., 1992) via an Excel macro (called ValiData). All quantitative analyses were computed considering the response ratios between analytes and internal standard. The performance and accuracy of the method were tested by standard addition experiments. The limit of detection (LOD) and limit of quantification (LOQ) for nicotine were found to be 3.0 and 10.8 pg, respectively. These correspond to 0.8 (LOD) and 2.7 μg kg^{-1} (LOQ) for fresh fruit assuming a 3 g sample. A standard deviation of regression of 4.3% was obtained. A detailed description of the development and validation of the methods has been given elsewhere (Siegmund et al., 1999).

RESULTS AND DISCUSSION

Analytical Methods. The determination of nicotine in food matrixes represents a demanding problem for the analytical chemist. Nicotine (Figure 1) is a tertiary base with pK_{a1} = 6.16 and pK_{a2} = 10.96 (Dash and Wong, 1996). Because most of the vegetables and fruits are acidic, nicotine is bound to the matrix as a salt. A second issue concerning nicotine determination at the low levels found in fruits and vegetables is the potential for contamination by airborne nicotine present in the environment as a constituent of environmental tobacco smoke. The development and validation of analytical techniques used in this study have been described and discussed in detail elsewhere (Siegmund et al., 1999).

Table 1. Nicotine Concentrations in Tomatoes of Different Varieties and Different Degrees of Ripening

variety	DR[a]	dry matter (%)	nicotine (μg kg^{-1}) ww [b]	nicotine (μg kg^{-1}) dw [c]	SD (%) (n=4)
Culina	1	6.7	16.1	240.9	11.8
Culina	3	5.4	5.6	103.4	11.2
Culina	7−8	5.8	2.8	48.4	11.0
Culina	12	5.7	nq[d]	nq[d]	
Mercedes	1	8.2	7.6	92.0	8.7
Mercedes	3	6.0	nq[d]	nq[d]	
Mercedes	7−8	4.7	3.5	73.2	9.0
Mercedes	10	5.5	nq[d]	nq[d]	
Mercedes	12	5.0	2.7	41.1	15.8
Furore	1	6.8	5.9	86.8	4.4
Furore	3	6.3	nq[d]	nq[d]	
Furore	7	6.9	3.9	56.4	20.7
Furore	9	6.5	3.3	51.4	4.7
Furore	11	6.2	2.9	47.2	6.5
Favorita	1	8.5	7.2	84.8	13.4
Favorita	3−4	7.0	2.7	37.8	6.1
Favorita	7−8	7.1	3.2	45.3	12.0
Favorita	9−10	7.3	3.1	42.1	17.9
Favorita	12	8.8	3.9	44.1	15.6
Marinda	1	6.8	4.4	64.8	0.4
Marinda	3−4	6.5	4.8	73.1	3.9
Marinda	8−9	6.2	nq[d]	nq[d]	
Marinda	12	6.1	nq[d]	nq[d]	
Hypeel 108	1	8.7	7.3	84.4	12.1
Hypeel 108	4	6.4	nq[d]	nq[d]	
Hypeel 108	8	7.3	3.2	43.8	7.3
Hypeel 108	11	5.4	nq[d]	nq[d]	
Alteza	1	7.0	7.6	107.6	1.7
Alteza	3−4	5.8	nq[d]	nq[d]	
Alteza	7−8	4.7	nq[d]	nq[d]	
Alteza	12	6.1	2.7	44.7	1.9

[a] DR = degree of ripening. [b] ww = wet weight. [c] dw = dry weight. [d] nq = the nicotine concentration was between LOD and LOQ and, therefore, not quantifiable.

Nicotine Concentrations in Different Matrixes. *Tomatoes.* Seven commonly grown varieties of tomatoes were investigated by following nicotine concentrations in the fruit during the course of the ripening process. This study was conducted for two reasons. First, in some countries the consumption of green unripe tomatoes in the form of fried or pickled tomatoes is very common. Second, frequently transportation of tomatoes after the harvest requires that the fruits are picked before they are fully ripe and are, therefore, usually harvested in a partially ripened stage (according to a degree of ripening of 6−8). Table 1 shows the nicotine concentrations of the tomatoes that were investigated. Nicotine was detected in all tomatoes up to a concentration of about 16 μg kg^{-1} of fresh fruit (wet weight), but there were a few samples showing concentrations lower than the LOQ. A clear relationship between nicotine concentrations and the degree of ripening was found. This is illustrated with the variety *Culina*, as shown in Figure 2. A rather high nicotine concentration was found in the unripe, green fruits. With increasing ripeness, the nicotine concentration decreased to a more or less constant range of 2−4 μg kg^{-1} of fresh fruit. It is important to note that there is no significant difference in concentration between fruits from DR 7 to DR 12 and that fruits from these ripening stages cover the major part of tomatoes on the market. The other varieties investigated showed comparable nicotine concentrations. However, the dependence on the degree of ripening is not as distinctive as with *Culina*, which showed

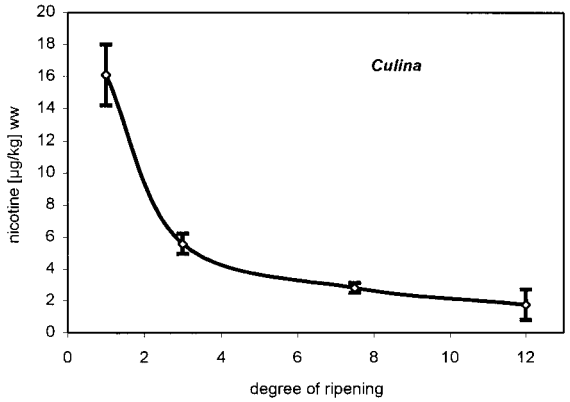

Figure 2. Nicotine concentration of the variety *Culina* depending on the degree of ripening; as the nicotine concentration at DR 12 was <LOQ, the nicotine concentration was calculated as (LOD + LOQ)/2; for the calculation of SD, LOD/LOQ values were set as lower and upper values; $n = 4$ in each ripening stage.

the highest concentration in the green stage. Nevertheless, all investigated green unripe fruits showed significantly higher nicotine values than tomatoes of higher ripening stages. These results could possibly confirm the assumption that the plants produce nicotine as a natural defense against insects or animals (Ames, 1983; Kuhn, 1964).

The results reported here compare well with those of Davis et al. (1991) and Domino et al. (1993), who reported 4.08 μg kg^{-1} and 5.1 as well as 9.8 wet weight, respectively for nicotine in tomatoes. Castro and Monji (1986) also reported values in a similar concentration range for ripe tomatoes and gave the first indication that the nicotine concentration could decrease in the course of the ripening process. Differences are also shown within different varieties of tomatoes. The results reported here also show that (1) green tomatoes of different varieties do not obtain consistent nicotine concentrations in the unripe stage but (2) show significantly lower as well as consistent values for the fully ripe stage for all tomato varieties investigated.

Data reported by Sheen (1988) are on a dry weight basis. Nevertheless, the values reported there are significantly higher than all other reports. In comparison to our results for ripe tomatoes, the values reported by Sheen (1988) are a factor of 10 higher, even if corrected to a typical wet weight basis. Davis et al. (1991) have previously questioned these results.

Tomato Products. There has been no systematic study concerning processed products from tomatoes with regard to nicotine content. Only two citations refer to nicotine concentrations in tomato products. Castro and Monji (1986) report values for tomato paste and tomato sauce, which are in the same concentration range as the values found in our laboratory. Surprisingly, they report one kind of peeled tomatoes with a nicotine concentration of 52 μg kg^{-1}, which is not in agreement with the values reported for their fresh whole fruits and which is higher by a factor of 10 for the peeled tomatoes that we examined. Consequently, the question is posed as to whether this high concentration is genuine from tomatoes used for this product or if this sample was potentially contaminated during harvesting, processing, or analysis. Sheen (1988) investigated nicotine concentration in tomato products. In contrast to the relatively high concentrations reported for fresh fruits, no nicotine was found in tomato ketchup or tomato paste.

Table 2. Nicotine Concentration in Various Products of Processed Tomatoes

sample	dry matter (%)	nicotine (μg kg^{-1}) ww	nicotine (μg kg^{-1}) dw	SD (%) ($n = 4$)
PT 1[a]	5.4	nq[i]	nq[i]	
PT 2[a]	5.0	3.3	21.9	20.1
PT 3[a]	6.3	4.4	70.6	42.7
PT 4[a]	6.1	nq[i]	nq[i]	
HT 1[b]	8.9	4.5	50.4	13.4
HT 2[b]	8.6	6.3	73.8	10.3
HT 3[b]	9.4	6.4	68.5	17.2
TS 1[c]	11.7	5.7	48.4	8.2
TS 2[d]	17.2	6.2	35.8	10.1
TS 3[e]	15.9	5.1	32.0	1.2
TS 4[f]	19.4	4.5	22.9	4.8
TP 1[g]	41.4	17.5	42.3	6.9
TP 2[g]	32.2	76.4	237.4	1.4
TP 3[g]	38.6	8.6	22.3	11.4
TP 4[g]	31.4	99.0	315.2	10.5
TP 5[g]	30.5	13.0	42.7	12.3
TK 1[h]	39.4	6.4	16.1	4.1
TK 2[h]	36.3	5.8	16.0	2.2
TK 3[h]	35.4	6.4	18.2	2.4
TK 4[h]	37.8	10.3	27.4	3.2
TK 5[h]	37.6	7.8	20.6	11.3
TK 6[h]	40.0	7.0	17.6	13.3

[a] Peeled tomatoes sold in cans. [b] Peeled and homogenized tomatoes sold in cardboard boxes. [c] Tomato sauce with olives, capers, and herbs. [d] Tomato sauce with vegetables. [e] Tomato sauce with basil. [f] Tomato sauce with different spices. [g] Concentrated tomato pulp sold in tubes, cans, or glasses. [h] Tomato ketchup sold in glass or plastic bottles. [i] nq = the nicotine concentration was between the LOD and LOQ and, therefore, not quantifiable.

In this investigation, a number of commercially available products were analyzed for their nicotine concentration. These investigations do not cover the entire range of tomato products available on the market. The purpose of conducting this part of the study was (1) to determine whether there is nicotine present in tomato products indicating its survival during processing and (2) to check if the nicotine concentrations vary in a large amount within one group of products.

The nicotine concentrations found in the processed products are shown in Table 2. All products investigated showed detectable nicotine concentrations, even products that are thermally processed, which indicates that the analyte is thermally stable and does not degrade or evaporate during processing. A slight increase in nicotine concentration over fresh tomatoes was found in examples such as peeled tomatoes sold in cans (PT), homogenized tomatoes (HT), and commercially available tomato sauce (TS). The observed increase in concentration within these three groups of products is related to the decrease of the water content. Within these product groups the observed values are more or less consistent. Nicotine concentrations for canned peeled tomatoes are low but in the same range as fully ripe tomatoes. Values for concentrated tomato pulp (TP) do not show a definite correlation to the increasing dry matter nor are the concentrations consistent within this group, ranging over a factor of 10. The nicotine content of tomato ketchup (TK) is larger than fresh tomatoes and consistent within the group but does not show correlation with the water content when compared to fresh tomatoes.

Potatoes. Potatoes are among the most widely consumed vegetables. Four different commonly consumed varieties from two different harvest years were investigated. The results are listed in Table 3. Nicotine was detected in all samples investigated, but again the

Table 3. Nicotine Concentration of Different Varieties of Raw Potatoes

sample	variety no./ harvest year	dry matter (%)	nicotine (μg kg^{-1}) ww	nicotine (μg kg^{-1}) dw	SD (%) ($n = 4$)
P1	I/1998	17.2	nq[a]	nq[a]	
P2	II/1997	21.6	3.3	15.1	21.4
P3	III/1997	19.6	7.6	38.9	7.3
P4	II/1998	18.6	5.7	30.5	11.7
P5	IV/1998	10.0	3.5	35.4	14.8
P6	III/1998	22.2	5.3	23.9	11.9
P2[b]	III/1997	23.9	4.6	19.4	9.7
P4[b]	II/1998	25.0	nq[a]	nq[a]	
P5[b]	IV/1998	19.7	3.8	19.1	24.7
P6[b]	III/1998	20.1	3.3	16.2	12.5
FF 1[c]		50.9	11.5	22.6	4.5
FF 2[c]		33.6	6.9	20.7	12.7

[a] nq = the nicotine concentration was between the LOD and LOQ and, therefore, not quantifiable. [b] Potatoes were cooked under atmospheric pressure, peeled, and analyzed. [c] French fries.

Table 4. Nicotine Concentrations in Different Varieties of Aubergines

sample	dry matter (%)	nicotine (μg kg^{-1}) ww	nicotine (μg kg^{-1}) dw	SD (%) ($n = 4$)
A1[a]	6.4	2.9	44.7	8.5
A2[b]	12.0	nd[e]	nd[e]	
A3[c]	10.1	nq[f]	nq[f]	
A4[d]	9.0	nq[f]	nq[f]	

[a] Common type purchased in a local supermarket. [b] Common type, obtained from the breeding station. [c] *Pintung Long*, Chinese breeding. [d] *Ichiban*, Turkish breeding. [e] Not detectable. [f] nq = the nicotine concentration was between the LOD and LOQ and, therefore, not quantifiable.

concentrations were low. No significant differences could be observed between samples of different varieties or different harvesting years.

Three prior publications were found which discuss the presence of nicotine in potatoes. Domino et al. (1993) reported 7.12 μg kg^{-1} (wet weight), which is slightly higher than the values found in our laboratory. Sheen (1988) as well as Davis et al. (1991) investigated potato peel and flesh separately. Sheen (1988) did not detect nicotine in the flesh but found a rather high concentration in the peel. The question arises whether surface contamination of the potato led to high values. Davis et al. (1991) reported nicotine concentrations for potato flesh in the same range as our results but with somewhat higher concentrations in flesh than in the peel, which is not in agreement with the results obtained by Sheen (1988).

Processed Potatoes. No publications were found concerning processed or cooked potatoes, which is rather surprising as potatoes are not generally consumed as a raw vegetable. In this study, we investigated cooked potatoes as well as French fries, two products that represent the most frequently consumed forms of potatoes. Table 3 shows the results. Nicotine was observed in all samples investigated. The nicotine concentrations observed in the cooked potatoes do not differ significantly from the raw potatoes. This again indicates that nicotine is thermally stable and does not evaporate during processing. The French fries showed slightly higher amounts of nicotine that can again be interpreted as a result of the higher percentage of dry matter.

Aubergines. Aubergines were investigated by Castro and Monji (1986), Sheen (1988), and Davis et al. (1991) with inconsistent results. The concentrations published previously include not detectable (Davis et al., 1991), >100 μg kg^{-1} wet weight (Castro and Monji, 1986), and 2.65 mg kg^{-1} dry weight (Sheen, 1988). We investigated four different types of aubergines, and nicotine could only be quantified in one sample. In two of the remaining samples, nicotine was detected but could not be quantified (Table 4). Overall, the results are most comparable to those obtained by Davis et al. (1991).

Table 5. Nicotine Concentration in Diverse Peppers

sample	dry matter (%)	nicotine (μg kg^{-1}) ww	nicotine (μg kg^{-1}) dw	SD (%) ($n = 4$)
PE 1[a]	5.0	3.7	73.0	15.7
PE 2[b]	12.1	5.9	48.4	4.0
PE 3[c]	5.6	5.8	103.5	20.1
PE 4[d]	5.5	6.1	110.7	5.7
PE 5[e]	6.0	9.0	149.1	1.3
PE 6[f]	8.5	8.7	102.1	2.9
PE 7[g]	11.0	6.3	57.6	13.4

[a] Green pepper, purchased in a local supermarket. [b] Red pepper, purchased in a local supermarket. [c] *Bendigo*, light green pepper. [d] *Multi*, dark green pepper. [e] *Flamingo*, yellow pepper. [f] Dark green, hot pepperoni. [g] Light green, mild pepperoni.

Table 6. Nicotine Concentrations in Tea Leaves

sample	dry matter (%)	nicotine (μg kg^{-1}) ww	nicotine (μg kg^{-1}) dw	SD (%) ($n = 4$)
tea 1[a]	95.0	381.0	403.9	13.6
tea 2[b]	94.0	163.8	174.3	22.1
tea 3[c]	94.0	1,593.1	1,695.7	4.1
tea 4[d]	93.9	811.6	864.0	11.2
tea 5[e]	94.0	317.2	337.3	3.3
tea 6[f]	94.1	358.0	380.3	0.2
tea 7[g]	94.0	469.6	499.4	3.3
tea 8[h]	95.4	336.8	353.0	3.6

[a] Black tea, Earl Grey. [b] Black tea, Ceylon Orange Pekoe. [c] Black tea, Assam. [d] Black tea, Darjeeling. [e] Green tea, China Fancy Gunpowder. [f] Green tea, Earl Grey. [g] Green tea, Formosa Gunpowder. [h] Green tea, Temple of Heaven.

Peppers. The last group of Solanaceae investigated are a variety of peppers, including sweet peppers and pepperonis. All varieties observed showed detectable but low nicotine concentrations (Table 5). These results compare well to data from the literature. Davis et al. (1991) investigated one kind of pepper and did not detect nicotine. Castro and Monji (1986) examined two types of green peppers and found similar nicotine concentrations in each. In contrast, the data reported by Sheen (1988) are 1−2 orders of magnitude higher.

Tea. Conflicting results are found in the literature concerning nicotine concentrations in black tea (Sheen, 1998; Davis et al., 1991; Domino et al., 1993). Therefore, tea samples were analyzed for nicotine to address these contradictory results. The concentrations that were found in the dry tea leaves (Table 6) were surprisingly high in concentration, ranging from 163 to 1600 μg kg^{-1}. Large variations were found within the types of black tea, whereas the concentrations were more or less consistent within the green teas. For an estimation of the dietary nicotine intake from tea, the nicotine concentration of the tea leaves is less relevant than that in brewed tea. Tea was brewed using common amounts of tea leaves and water. The results show that nicotine is not efficiently extracted by conventional brewing techniques (Table 7). Even tea with very high nicotine concentrations in the leaves (e.g., teas 3 and 4) do not show high amounts in the brewed tea. If detectable, the extraction yield is in a range of 20−25%.

The results observed in our laboratory may also explain the contradictory values reported in the litera-

Table 7. Nicotine Concentrations in Brewed Tea

sample [a]	nicotine in brewed tea (μg L^{-1})	nicotine extracted from the leaves (%)
tea 1	nd[b]	
tea 2	nq[c]	
tea 3	4.2	21.3
tea 4	3.8	27.7

[a] Numbers refer to Table 7. [b] nd = not detectable. [c] nq = not quantifiable.

Table 8. Average Consumption Data for Potatoes, Tomatoes, Tomato Paste, Aubergines, and Brewed Tea

daily consumption	average (g/day)	SD (g/day)
potato consumption	157	80
tomato consumption	33	21
tomato paste consumption	20	26
aubergine consumption	1.4	3.8
tea consumption (brewed)	142	28

ture. Sheen (1998) again reported very high concentrations, still at least 1 order of magnitude away from our results. Davis et al. (1991) found nicotine in some tea samples, reporting rather high nicotine concentrations in brewed tea (mean 69 μg L^{-1}). Domino et al. (1993) could not detect any nicotine in tea. The results summarized in Table 6 in comparison to data found in the literature show that nicotine is not present in tea in consistent amounts. The question of the origin of nicotine in tea cannot be determined.

Nicotine content in brewed tea both reported here and by Davis et al. (1991) is not consistent. For the calculation of the dietary nicotine intake, we elected to use the mean and standard deviation of the results obtained in this work for brewed tea made from regular tea leaves, disregarding instant and decaffeinated teas. We calculate this to be 4.0 $\pm$ 0.3 ng/g of nicotine in brewed tea. This is about an order of magnitude lower than reported by Davis et al. (1991).

Dietary Nicotine Intake. *Consumption Data.* The results obtained in this study provide an opportunity to estimate the dietary intake of nicotine assuming that adequate food consumption data are available. Such data are available from a variety of governmental services and are, in a few cases, summarized in publications. These data are most often based on crop production corrected for import/export or on 24-h recall questionnaire data. In most cases, vegetables are grouped into categories and only vegetables consumed in large quantity are listed separately. Essentially no consumption information is available on processed products such as tomato ketchup or sauces. Any attempt to estimate consumption of vegetables and their products will be subjected to these limitations.

Table 8 shows the consumption data used to estimate daily dietary nicotine intake. These four vegetables make up the vast majority of vegetables consumed that are known to contain nicotine. The data shown in Table 8 were collected from a variety of sources (TNO, USDA, Grüter et al., 1998) and represent the mean and standard deviation of the daily per capita consumption of the vegetables and products shown for 13 European countries (Austria, Belgium, Denmark, Great Britain, Finland, France, Germany, Italy, Netherlands, Portugal, Spain, Sweden, Switzerland) and the United States.

Concerning tea consumption data, Chappell and Gratt (1996) reported an average consumption of brewed tea of 142 g/day with a very erratic distribution. An estimate of a standard deviation of 20% is difficult to justify. Nonetheless, we selected that value for use in the simulation described below.

Table 9. Averaged Nicotine Concentrations Based on the Observed Nicotine Concentrations[a]

source	nicotine (ng/g)	SD (ng/g)
nicotine from potatoes[b]	4.5[j]	1.9
nicotine from tomatoes[c]	2.7[g]	0.7
nicotine from tomato paste[d]	5.3[j]	0.6
nicotine from tomato sauce[e]	4.5	1.5
nicotine from ketchup[f]	7.3	1.5
nicotine from aubergine[g]	2.1[j]	0.5
nicotine from brewed tea[i]	4.0[j]	0.3

[a] For products with nicotine concentrations below the LOQ, but above the LOD, the concentration was set 1.75 μg kg^{-1} (corresponding to (LOD + LOQ)/2); for products with no detectable nicotine, the concentration was set according to the LOD (0.8 μg kg^{-1}). [b] Referred to the values obtained from raw potatoes (Table 3). [c] Referred to tomatoes of DR > 7−8 (Table 1). [d] Referred to ready-to-eat tomato sauce (TS in Table 2). [e] Referred tomato sauce to products that are used for the preparation of tomato paste (PT, HT, and TS in Table 2). [f] Referred to TK in Table 2. [g] Referred to Table 4. [i] Referred to concentrations reported in Table 7. [j] Values used in estimation of nicotine intake.

Table 9 shows the average and standard deviation of the nicotine content of the fruits, vegetables, and their processed products as well as of brewed tea considered in this investigation. Because consumption data are not available for tomato sauce and tomato ketchup, these products were not used individually in the estimation of dietary nicotine intake. However, it is reasonable to assume that this is partially compensated for in reported total consumption of tomatoes.

Dietary Nicotine Intake. Using the data indicated in Tables 8 and 9, an average daily dietary intake of 1.4 μg/day of nicotine is calculated for the countries for which data are available. To obtain this result, the average consumption of each vegetable for all countries in which data are available is multiplied by the average nicotine content found for this vegetable. It is beyond the scope of this report to describe, in detail, the average dietary intake for each country. The highest levels are calculated for Italy and Portugal because of high consumption of tomatoes, aubergines, and potatoes. Lower values are estimated for France because of lower consumption of these vegetables. As stated earlier, these estimates are subject to the variations of nicotine content and the limitations of the consumption data that are available.

Monte Carlo Simulation. A point value such as the average daily nicotine intake for residents of the 14 countries represented in Table 8 does not provide information related to the probability distribution of the results. Given the mean and standard deviation, and assumptions concerning the upper and lower limits of the consumption data, a distribution can be simulated using a variety of mathematical approaches. One of these is Monte Carlo simulation, which can be performed on a desktop computer using commercial software such as the one used in this study (Crystal Ball).

In summary, a Monte Carlo approach begins with mean values of parameters, the standard deviation about those means, and some assumptions about the upper and lower boundaries of those values. In this case, the mean and standard deviation of the food consumption and nicotine content values are used. A lower limit of consumption of a food item is 0 g/day, indicating no consumption of that food. An upper limit of consumption

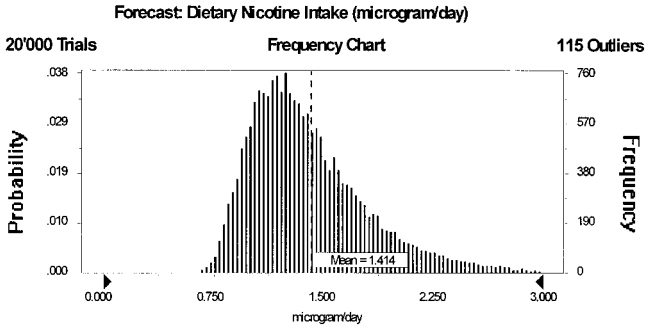

Figure 3. Estimated daily dietary nicotine intake based on Monte Carlo simulation.

was taken to be the value plus 3 times the standard deviation. A similar approach was taken for the nicotine content in each food substance. Considered together, these parameters and the statistical values associated with them make up a set of assumptions.

To perform a distribution simulation, one must either know or assume the nature of the distribution of the parameters used in the assumptions. For example, is the variation in the nicotine content in a particular vegetable distributed normally, log-normally, or some other type of distribution. If sufficient data is available, this can be tested. In this case, without sufficient data to conduct such a test, a log-normal distribution was assumed for both consumption and nicotine content of the vegetables. This type of distribution is frequently found when values cannot go below zero and have no practical upper limit. On the basis of the information available, it was decided that a log-normal distribution was the more appropriate one for these simulations.

The Monte Carlo method randomly and independently selects a set of parameters consistent with the assumptions described above. In this case, a number of grams/day of each food substance and a value for the nicotine content are selected. The product of these parameters is calculated and summed for all vegetables considered. This process is repeated a large number of times (typically 10000−20000), eventually resulting in a simulation of the probability distribution daily dietary nicotine intake of the population.

A Monte Carlo simulation using the data in Tables 8 and 9 is shown in Figure 3. This simulation estimates that a mean daily dietary intake for the population of the 14 countries is 1.4 μg/day. The upper 95th percentile is estimated to consume 2.25 μg/day. A sensitivity analysis indicates that potato consumption combined with the nicotine content of potatoes account for about 91% of the variance in daily nicotine intake. This is not an unreasonable result when the values in Tables 8 and 9 are considered.

The estimated nicotine concentrations derived from dietary sources with a maximum intake of 2.25 μg/day are low, if one compares this value to the nicotine amounts ingested by smoking—which are reported to be in the range of 1 mg per cigarette more or less independent of the brand and of the nominal nicotine rating (Benowitz and Jabob, 1984, 1994; Benowitz, 1996). Nevertheless, the direct comparison for ingested nicotine from any source is hard to perform, as not nicotine itself but its metabolites detected in biological fluids such as urine, blood serum, or saliva are generally used to estimate the exposure to environmental tobacco smoke (ETS).

The level of predicted salivary cotinine from dietary intake may be compared to that expected from exposure to ETS. Benowitz (1996) has reported that with light activity, breath ventilation rates are approximately 1 m^3/h, and approximately 71% of the inhaled nicotine is absorbed (Iwase et al., 1991). On the basis of personal monitoring studies in 16 U.S. cities, Jenkins (Jenkins et al., 1997) reports that the median 24-h time weighted average (TWA) exposure of subjects that are exposed to ETS in the workplace only but not away from work is 0.16 μg/m^3. Using these data, a median daily nicotine intake of 2.7 μg of nicotine from ETS exposure at work only is calculated. This value can be compared directly to the mean daily dietary nicotine intake of 1.4 μg reported here.

Nevertheless, further work is required to relate the estimated nicotine intake from dietary sources to nicotine metabolite concentrations in biological fluids to be able to make reliable statements about the importance of dietary nicotine in comparison to exposure to environmental tobacco smoke.

CONCLUSION

The edible Solanaceae analyzed in this investigation were found to contain relatively consistent amounts of nicotine in the range of 2−7 μg/kg for fresh fruits. These results are in agreement with most but not all of the previous results reported in the literature. Nicotine appears to survive a variety of processing operations such as the preparation of tomato ketchup, sauces, and pastes as well as frying and boiling of potatoes. These products showed slightly higher concentrations in comparison to the related fresh fruits. Relatively large concentrations of nicotine found in tea leaves were not reflected in brewed tea. Using food consumption data from government sources, a mean estimated daily dietary intake of nicotine is approximately 1.4 and 2.25 μg/day at the 95th percentile based on the nicotine content and consumption data discussed in this report. It is possible that these estimates are low because of incomplete food consumption data. Further work is required to relate the estimated dietary nicotine intake to nicotine metabolite concentrations in biological fluids to be able to make reliable statements about the importance of dietary nicotine intake in comparison to environmental tobacco smoke exposure.

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