JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Acute Toxicity of High Doses of the Glycoalkaloids, α-Solanine and α-Chaconine, in the Syrian Golden Hamster

SØREN LANGKILDE,[†] MALENE SCHRØDER,[†] DEREK STEWART,[‡] OTTO MEYER,[†] SEAN CONNER,[‡] HOWARD DAVIES,[‡] AND MORTEN POULSEN*,[†]

The National Food Institute (NFI), Technical University of Denmark, 2860-DK, Denmark, and Quality, Health and Nutrition Programme, Scottish Crop Research Institute (SCRI), Dundee, DD2 5DA, Scotland, United Kingdom

Sprouted, stressed, or spoiled potato tubers have reportedly led to human acute intoxication, coma, and death when consumed in high amounts. These effects have been attributed to glycoalkaloids (GAs), primarily α-solanine and α-chaconine, naturally present in all potatoes. The level of GAs in potato tubers has previously been shown to increase substantially as a result of improper handling and postharvest storage. A short-term study was performed to investigate the dose−response profile of α-solanine and α-chaconine alone or in combination, administered daily by oral gavage to Syrian Golden hamsters. Daily doses of 100 mg of α-solanine [kg body weight (BW)]$^{-1}$ induced death in two of four hamsters within 4 days, when administered by gavage to female Syrian hamsters. Doses of 100 mg of α-chaconine alone or α-solanine and α-chaconine combined in a ratio of 1:2.5, in doses of 75 or 100 mg (kg BW)$^{-1}$, induced death in one of four hamsters within the same period. Animals dosed with α-solanine alone or in combination with α-chaconine suffered from fluid-filled and dilated small intestines. The GA administration had no effect on acetyl cholinesterase (AChE) or butyryl cholinesterase (BuChE) activity in plasma or brain. Liquid chromatography−mass spectrometry-based metabolomics showed that there was a specific accumulation of α-chaconine in the liver tissues. In addition, metabolomics gave direct evidence of glycolytic metabolism of the GA with the β_1, β_2, and γ-GAs detected in the urine and, to a lesser extent, the feces. Doses from 75 mg (kg BW)$^{-1}$ of α-chaconine, α-solanine, or the two compounds combined were potentially lethal within 4−5 days in the Syrian Golden hamster. However, the cause of death in these studies could not be established. No synergistic effects of α-solanine combined with α-chaconine were evident.

KEYWORDS: Glycoalkaloid; α-solanine; α-chaconine; hamster; potato; acetyl cholinesterase; metabolomics

INTRODUCTION

Glycoalkaloids (GAs) are nitrogen-containing steroidal glycosides that are toxic compounds found in a number of vegetables and particularly in plants of the Solanaceae family, such as tomato (*Lycopersicon esculentum* L.) and potato (*Solanum tuberosum* L.) (*1*). In commercially bred potatoes, the major GA compounds are α-solanine and α-chaconine (*2*). The levels of GAs in potato can significantly alter following harvest, due to storage conditions, such as mechanical damage, temperature, light, and duration (*1, 3, 4*). These conditions significantly impact both the total GA content and the ratio of α-solanine:α-chaconine.

Multiple cases of poisoning in humans with symptoms, ranging from nausea, vomiting, diarrhea, and fever to delirium, coma, and death, have been related to the presence of GAs in potatoes (*5, 6*). Symptoms associated with the central nervous system, such as changes in pulse and breathing, delirium, or coma, have been attributed to the inhibition of cholinesterase activity. Symptoms, such as nausea, vomiting, cramps, and diarrhea, may be attributed to the cell membrane-disrupting properties of α-chaconine (*1*).

On the basis of the evaluation of cases related to the intake of GAs in potatoes, Morris and Lee (*7*) concluded that doses of 1−2 mg GA [kg body weight (BW)$^{-1}$] are marginally toxic to humans, and doses of 3−6 mg GA (kg BW)$^{-1}$ are potentially lethal. A more recent study by Hopkins (*8*) estimated that, in the United Kingdom at least, the daily per capita intake of potato-derived GAs was ∼14 mg, which for an individual with a weight of, for example, 60 kg, would represent a daily intake

* To whom correspondence should be addressed. Tel: +45 72347540. Fax: +45 72347698. E-mail: mop@food.dtu.dk.
[†] Technical University of Denmark.
[‡] Scottish Crop Research Institute (SCRI).

10.1021/jf8012794 CCC: $40.75
Published on Web 08/19/2008

of 0.23 mg (kg BW)$^{-1}$, well within the marginal toxic levels. However, a recent human intervention trial with GAs and cooked, mashed potato (*9*) reported that one subject who received a dose of GA of 1.25 mg (kg BW)$^{-1}$ (the highest dose in that study) became nauseous and started vomiting 4 h after consumption. This dose is at the lower end of the frequently quoted marginally toxic range of 1−2 mg GA (kg BW)$^{-1}$ (*7*). Interestingly, Mensinga et al. (*9*) also reported that clearance of GAs from the body was slow, with α-solanine and α-chaconine exhibiting half-lives of 21 and 44 h, respectively. This prolonged duration in the body implies the potential for persistent accumulation, particularly with respect to α-chaconine in those with regular and/or daily potato consumption.

Today, a safety limit of 200 mg total GA (kg tuber fresh weight)$^{-1}$ has been generally acknowledged (*10*), although surprisingly there are no EU legislative limits on GA levels in potato. Thus, the threshold between the accepted total amount of GAs in potatoes and the intake that causes toxicity in humans is relatively narrow.

A number of animal studies have been performed assessing the toxic effects of potato material and α-solanine and α-chaconine in the pure form on rats, mice, rabbits, and hamsters. The main effects observed from these studies are cytotoxic effects on cells and inhibition of acetyl cholinesterase (AChE) and butyryl cholinesterase (BuChE) activity in brain and plasma (*7, 11*−*15*). In addition to this, GAs have been suggested to be potentially damaging to embryos and fetuses (*16*).

Humans are more sensitive to the toxic effects of GAs than other mammals. However, several studies have shown that GAs are more readily absorbed across the intestinal epithelial barriers in hamsters as compared to other laboratory animals. The hamster is therefore considered the best animal model to investigate the toxicity of GAs (*11, 17*).

The present study in female Syrian hamsters included daily doses of GAs for 5 days and examined the acute adverse effects of α-solanine and α-chaconine, as previous studies do not provide sufficient information on the dose−response effects and toxicokinetics and cellular insults of these compounds. It was therefore decided to administer doses of 100 mg (kg BW)$^{-1}$ of α-solanine and α-chaconine or 75 or 100 mg (kg BW)$^{-1}$ of a mixture of α-solanine and α-chaconine in a ratio of 1:1.25, comparable to the ratio found in most commercially bred potatoes. Furthermore, blood, urine, feces, liver samples, and contents from the jejunum and stomach from animals dosed with 100 mg (kg BW)$^{-1}$ either α-chaconine or α-solanine were analyzed for metabolites.

MATERIALS AND METHODS

Preparation and Administration of Test Substances. The test substances (α-solanine and α-chaconine) were supplied by Sigma-Aldrich (St. Louis, MO) and kept at −20 °C until use. Both compounds had a claimed minimum purity of approximately 95%, but liquid chromatography−mass spectrometry (LC-MS) analysis showed this to be closer to 99%. α-Solanine was dissolved in H_2O containing 0.45% saline and 0.25% acetic acid (pH 3.0). α-Chaconine was dissolved in H_2O containing 0.45% saline and 0.28% acetic acid (pH 2.8). The vehicle used was H_2O containing 0.45% saline and 0.28% acetic acid (pH 2.8).

Animals and Housing. Twenty-four female Golden Syrian hamsters (HsdHan Aura), 4 weeks old, were bred at Harlan Netherlands B.V. Kreuzelweg 53 NL-5960 AD Horst and purchased from Harlan Scandinavia ApS Frederiksborgvej 71 DK-3450 Allerød. They were allowed to acclimatize for 15 days before the beginning of treatment. The animals were kept in Makrolon 3 cages (one animal/cage) with Aspen bedding material (TAPVEI OY, Fin-73620 Kortteinen, Finland). During the study, the temperature was maintained at 22 ± 1 °C, the relative humidity was 55 ± 5%, the air was changed 8−10 times/h, and light was on from 11:30 to 23:30. All animals were fed 7024 Altromin Hamster feed ad libitum and given drinking water with 0.175% citric acid (pH 3.1) ad libitum. Animal studies were performed under conditions approved by the Danish Agency for Protection of Experimental Animals and by the in-house Animal Welfare Committee.

Table 1. Study Design: The Control Group Consisted of Eight Hamsters Administered Vehicle Only—H_2O (0.45% Saline and 0.28% Acetic Acid, pH 2.8), and Test Groups Consisted of Four Animals

group	compound	GA dose mg (kg BW)$^{-1}$
1 (n = 8)	vehicle	0
2 (n = 4)	solanine:chaconine ratio, 1:2.5	75
3 (n = 4)	solanine:chaconine ratio, 1:2.5	100
4 (n = 4)	chaconine	100
5 (n = 4)	solanine	100

Dosing Procedure. Five groups of female Syrian Golden hamsters were given either α-solanine or α-chaconine alone in doses of 100 mg (kg BW)$^{-1}$ or in combination in a ratio of 1:2.5, α-solanine:α-chaconine (w/w), in doses of 75 or 100 mg (kg BW)$^{-1}$, by stomach tube for 5 consecutive days (**Table 1**). Animals in the control group were administered vehicle only. All hamsters were gavaged in the morning after weighing with 1 mL (100 g BW day)$^{-1}$ of either vehicle or test solution. Hamsters were monitored twice daily. Animals were weighed every day during the 5 day study. Food and water consumption were recorded for each animal at the beginning and end of the study. The relative daily food and water consumption were calculated for each animal.

Hematology, Blood Chemistry, and Pathology. At the end of the treatment periods, animals were anaesthetized by CO_2:O_2 (60:40) inhalation and sacrificed by decapitation. Blood was taken from the neck when decapitated and collected in ethylenediaminetetraacetic acid (EDTA)-coated Eppendorf tubes for hematology and heparin-coated tubes for clinical chemistry. Blood samples collected in EDTA tubes were analyzed using an Animal Blood Counter (ABC) Vet (ABX, France) for the following parameters: total leukocyte count (wbc), erythrocyte count (rbc), platelets (plt), hemoglobin (hgb), hematocrit (hct), mean cell volume (mcv), mean corpuscular hemoglobin (mch), and mean corpuscular hemoglobin concentration (mchc). Plasma was analyzed for urea, alanine transaminase, bilirubin, sodium, potassium, cholesterol, protein, albumin, creatine, alkaline phosphatase, and glucose using a Hitachi 912 Clinical Chemistry System (Roche Diagnostics GmbH, Mannheim, United States). All kits were purchased from Roche Diagnostics GmbH. The brain was dissected free, weighed, and homogenized in 10 mL of ice-cold 0.32 M sucrose. AChE and BuChE activities were measured on brain extract and plasma using a Hitachi 912 Clinical Chemistry System and basically followed the method of Kluge et al. (*18*). All substrates were made in-house. The AChE activity was measured using an acetylthiocholine iodide substrate (156 mM) with a phosphate buffer (52 mM, pH 7.2) containing 5,5′-dithio-bis[2-nitrobenzoic acid]) (0.26 mM). The BuChE activity was measured using a butyrylthiocholine iodide (218 mM) substrate with a phosphate buffer (52 mM, pH 7.7) containing 5,5′-dithio-bis[2-nitrobenzoic acid] (0.26 mM).

All animals were subjected to macroscopic examination. Tissue samples from each animal were dissected free of adherent fat and loose connective tissue. Abnormalities were noted, and the weights of liver, kidney, adrenal glands, and brain were recorded. Colon, small intestines, liver, kidney, and adrenal glands were preserved in 4% buffered formaldehyde for a minimum of 24 h. For histopathology, the organs were embedded in paraffin in sections of 4−6 μm and stained routinely with hematoxylin−eosin (H&E) for light microscopy. From the jejunum, frozen sections were made and stained with Oil Red O, following a standard protocol for this lipid staining.

GA and Metabolite Analysis. Additional hamsters (n = 2) were dosed with either 100 mg of α-solanine or 100 mg of α-chaconine (kg BW)$^{-1}$ for metabolite analyses in the same way as previously described. These animals were kept in metabolism cages for 24 h following the first dose. Feces and urine samples were collected and kept at −80 °C

Table 2. Mean Weight Gain/Loss [Gram (Day)$^{-1}$], Mean Relative Food Consumption [Gram (Day)$^{-1}$], and Mean Relative Water Consumption [Gram (Day)$^{-1}$][a]

	vehicle ($n = 8$)	solanine:chaconine 1:2.5; 75 mg (kg BW)$^{-1}$ ($n = 3$)	solanine:chaconine 1:2.5; 100 mg (kg BW)$^{-1}$ ($n = 3$)	chaconine 100 mg (kg BW)$^{-1}$ ($n = 3$)	solanine 100 mg (kg BW)$^{-1}$ ($n = 2$)
weight gain/loss, days 1−5	2.0 ± 2.5	−1.3 ± 4.5	−8.3 ± 7.3[b]	−4.0 ± 5.2[b]	−6.5 ± 4.1[b]
relative food consumption, days 1−5	161 ± 19	102 ± 53[b]	93 ± 37[b]	101 ± 67	70 ± 18[b]
relative water consumption, days 1−5	170 ± 31	187 ± 9	292 ± 79[c]	165 ± 54	272 ± 93[c]

[a] Values are means ± SD. [b] Statistical significance is lower ($P \leq 0.05$) than the control group given vehicle as assessed by the Wilcoxon test. [c] Statistical significance is higher ($P \leq 0.05$) than the control group given vehicle as assessed by the Wilcoxon test.

Table 3. Organ Weight Relative to BW [Gram Organ (kg BW)$^{-1}$][a]

	vehicle ($n = 8$)	solanine:chaconine 1:2.5; 75 mg (kg BW)$^{-1}$ ($n = 3$)	solanine:chaconine 1:2.5; 100 mg (kg BW)$^{-1}$ ($n = 3$)	chaconine 100 mg (kg BW)$^{-1}$ ($n = 3$)	solanine 100 mg (kg BW)$^{-1}$ ($n = 2$)
liver	47.3 ± 3.8	40.8 ± 3.0[c]	39.1 ± 4.1[c]	37.1 ± 6.7[c]	34.7 ± 2.0[c]
kidney	11.6 ± 0.5	11.2 ± 0.5	12.5 ± 1.7	11.5 ± 1.2	11.6 ± 2.4
adrenal gland	0.154 ± 0.026	0.181 ± 0.035	0.330 ± 0.234[b]	0.222 ± 0.118	0.179 ± 0.009

[a] Values are means ± SD. [b] Means are significantly higher ($P \leq 0.05$) in treatment groups as compared to controls, as determined by the Wilcoxon test. [c] Means are significantly lower ($P \leq 0.05$) in treatment groups as compared to controls, as determined by the Wilcoxon test.

until analyzed. Blood was sampled from the orbital vein 24 h after the first dosage and stored in EDTA-coated tubes at −80 °C. Furthermore, samples of the liver and content of jejunum and stomach were collected at euthanization after 5 days. Biofluids and tissues were subject to a "universal" extraction procedure developed at SCRI. This method (*19, 20*) uses a mixed chloroform, methanol, and water extraction stem that yields two fractions, which contain polar (aqueous/methanol) and nonpolar (chloroform) metabolites [small molecular weight (<2 kDa)]. Briefly, samples were analyzed on a LCQ-DECA system (Thermo, Hemel Hempstead, United Kingdom), comprised of a Surveyor autosampler, MS-pump and photodiode array detector (PDA), and an iontrap mass spectrometer. Chromatography was performed on a C_{18} Synergi Hydro RP column (4 μm, 2 mm × 150 mm; Phenomenex, Macclesfield, United Kingdom), and the mobile phase used for the separation of crude samples was a linear gradient spanning 35 min of 0.2% formic acid in deionized water to 0.2% formic acid in 90% acetonitrile at a flow rate of 200 μL min^{-1}. The sample injection volume was 10 μL, and the re-equilibration time was 10 min. Metabolite analyses employed electrospray ionization−mass spectrometry (ESI-MS) in positive and negative modes, using the following MS conditions; sheath gas (N_2), 70 psi; auxiliary gas, 15 psi; spray voltage, 4500 V; and capillary temp, 250 °C. The ions, specific for analyses, were used as follows; α-solanidine, *m/z* 868.8 $[M + H]^+$; and α-chaconine, *m/z* 852.7 $[M + H]^+$. β_{1and2}- and γ-Chaconine and solanine were generated by acid hydrolysis of the parent compounds (α-GAs), as described by Lee et al. (*21*), and then purified using the chromatographic conditions described above. These purified β_{1and2}- and γ-GAs were used for confirmation of structure in the chromatographic analysis of the tissue and fluids extracted as described above. Quantification of α-solanine and α-chaconine was determined using calibration curves ranging from 833.3 to 3.2 μg L^{-1} of GAs. r^2 values were 0.992 (α-solanine) and 0.993 (α-chaconine). The detection limits using this method were 0.3 μg L^{-1} with a reproducibility of ±11%. Hydrolysis products of α-solanine and α-chaconine were produced by acid-catalyzed hydrolysis of commercially available α-solanine and α-chaconine (Sigma-Aldrich, Poole Dorset) (*22, 23*).

Statistical Analysis. Data were tested for homogeneity of variance between groups and for normality distribution. If data were not normally distributed, a nonparametrical test was performed, using Kruskal−Wallis followed by the Wilcoxon test. $P \leq 0.05$ was considered significant. Metabolomic data were analyzed using principle component analysis (PCA).

RESULTS

Clinical Effects. Very few clinical signs were detectable on the first 3 days of dosing. In the group of hamsters given 100 mg α-solanine (kg BW)$^{-1}$, two animals died on day four. Besides this, one of the animals given the combination of α-solanine and α-chaconine in a total of 75 mg GA (kg BW)$^{-1}$ had severe symptoms of distress, such as piloerection and poor balance, and the animal was euthanized on day four. On the morning of day five, one animal from the group given 100 mg α-chaconine (kg BW)$^{-1}$ was found dead. Thirty minutes after dosing on day five, one animal, given a ratio of α-solanine and α-chaconine in a total of 100 mg GA (kg BW)$^{-1}$, was found dead. The cause of death for this animal was considered a consequence of incorrect administration into the lungs.

BW, Food Consumption, and Water Consumption. Dosed animals from all dose groups had a mean weight loss within the treatment period, whereas control animals gained weight. For the animals given α-solanine and α-chaconine alone and the high dose of combined α-solanine and α-chaconine, the BW was statistically significantly different from the controls. Dosed animals had statistically significantly lower relative food consumption as compared to controls from days 1 to 5, except for the 100 mg α-chaconine (kg BW)$^{-1}$ group as compared to controls ($p = 0.0511$). Animals administered 100 mg (kg BW)$^{-1}$ α-solanine alone had statistically significant higher relative water consumption than controls. Animals given 100 mg α-solanine and α-chaconine (kg BW)$^{-1}$ combined had statistically significantly higher relative water consumption as compared to both controls and animals given the low combined dose (**Table 2**).

Organ Weight. A statistically significant decrease in relative liver weight was observed for all dose groups as compared to controls (−27%). Dosed animals had higher relative adrenal gland weight as compared to controls; however, only statistically significant effects were observed for the high combined dose (+114%) (**Table 3**).

Macroscopic Pathology and Histopathology. One of four animals in each of the groups given α-solanine and α-chaconine combined to a total of 75 or 100 mg (kg BW)$^{-1}$ suffered from notable dilation of their small intestines, which were often filled with foamy fluids (**Figure 1**). All animals given 100 mg of α-solanine alone had the same symptoms. None of the four animals given α-chaconine alone had distended intestines. In some animals, hyperemia was observed in both the stomach and the small intestines, and a change in liver texture was noted in a few dosed animals. The change in texture of the liver was not supported by any histological findings.

On histopathological examination, large dilated cells/vacuoles were observed on the tip of the villi only in the

Table 4. Hematology Parameters from Blood Samples Taken at Euthanization on Day 5[a]

	vehicle ($n = 8$)	solanine:chaconine1:2.5; 75 mg (kg BW)$^{-1}$ ($n = 3$)	solanine:chaconine 1:2.5;100 mg (kg BW)$^{-1}$ ($n = 3$)	chaconine 100 mg/kg BW mg (kg BW)$^{-1}$ ($n = 3$)	solanine 100 mg (kg BW)$^{-1}$ ($n = 2$)
wbc ($\times 10^9$/L)	8.9 ± 3.9	10.4 ± 3.5	5.6 ± 1.1	9.0 ± 1.6	8.5 ± 3.5
rbc ($\times 10^{12}$/L)	7.8 ± 1.1	8.8 ± 0.5	9.6 ± 0.4[b]	9.2 ± 0.3[b]	10.2 ± 0.1[b]
plt ($\times 10^9$/L)	452.8 ± 91.4	416 ± 44.2	492.3 ± 18.5	447.8 ± 40.4	593 ± 94.8
hgb (mmol/L)	14.8 ± 2.1	16.7 ± 0.6[b]	18.4 ± 0.9[b]	17.4 ± 0.7[b]	19.2 ± 0.2[b]
hct (%)	46.5 ± 6.1	52.6 ± 2.5[b]	57.8 ± 3.2[b]	54.9 ± 2.5[b]	60.0 ± 0.6[b]
mcv (fl)	59.8 ± 1.3	60 ± 1.0	60.3 ± 0.5	59.8 ± 0.5	59 ± 0.0
mch (fmol)	19.1 ± 0.5	19.0 ± 0.4	19.2 ± 0.1	18.9 ± 0.2	18.9 ± 0.3
mchc (mmol/L)	31.9 ± 0.6	31.7 ± 0.4	31.8 ± 0.3	31.7 ± 0.1	32.0 ± 0.6

[a] Values are means ± SD. [b] Means are significantly higher ($P \leq 0.05$) in treatment groups as compared to controls, as determined by the Wilcoxon test.

Table 5. Clinical Chemistry Parameters from Blood Samples Taken at Euthanization on Day 5[a]

	vehicle ($n = 8$)	solanine:chaconine 1:2.5; 75 mg (kg BW)$^{-1}$ ($n = 3$)	solanine:chaconine 1:2.5; 100 mg (kg BW)$^{-1}$ ($n = 3$)	chaconine 100 mg (kg BW)$^{-1}$ ($n = 3$)	solanine 100 mg (kg BW)$^{-1}$ ($n = 2$)
urea (mmol/L)	8.1 ± 0.8	9.4 ± 0.6	9.1 ± 0.6	8.5 ± 0.7	11.4
alanine aminotransferase (u/L)	91 ± 17	83 ± 5	89 ± 18	87 ± 29	88
sodium (mmol/L)	139 ± 1	141 ± 2	145 ± 1[b]	143 ± 4[b]	144 ± 3[b]
potassium (mmol/L)	11.8 ± 1	12.6 ± 3.4	12.2 ± 1.1	12.0 ± 0.5	11.6 ± 1
cholesterol (mmol/L)	3.0 ± 0.4	2.7 ± 0.7	2.5 ± 0.1	2.9 ± 0.7	3.8 ± 0.6
protein (g/L)	54 ± 2	54 ± 6	55 ± 4	55 ± 4	57 ± 3
albumin (g/L)	25 ± 1	23 ± 2	22 ± 3	22 ± 1[c]	18 ± 2[c]
creatine (ymol/L)	6.0 ± 0.5	6.0	8.7 ± 1.5[b]	11.0 ± 3.8[b]	16.5 ± 0.7[b]
alkaline phosphatase (u/L)	661 ± 75	594 ± 58	499 ± 122	600 ± 106	502 ± 100[c]
glucose (mmol/L)	8.7 ± 1.4	8.4 ± 1.2	8. ± 1.2	8.8 ± 5.3	11.8 ± 1.1[b]

[a] Values are means ± SD. [b] Means are significantly higher ($P \leq 0.05$) in treatment groups as compared to controls, as determined by the Wilcoxon test. [c] Means are significantly lower ($P \leq 0.05$) in treatment groups as compared to controls, as determined by the Wilcoxon test.

jejunum; these were identified as fat-containing epithelial cells. However, the changes were also observed in some control animals.

Hematology. Statistically significant increases in rbc, hgb, and hct were seen in all dosed groups as compared to controls, except for rbc in the dose group of 75 mg (kg BW)$^{-1}$ (**Table 4**).

Blood Chemistry. Levels of sodium were statistically significantly higher in animals dosed with 100 mg of α-chaconine or α-solanine alone or combined as compared to controls (**Table 5**). Levels of albumin were significantly lower in groups dosed 100 mg of α-chaconine or α-solanine as compared to controls. Alkaline phosphatase was statistically significantly lower in animals given α-solanine alone. In the other dose groups, there were similarly decreased levels, but these changes were not statistically significant. Creatine was statistically significantly higher in animals given α-solanine or α-chaconine alone or the high combined dose, as compared to controls. Glucose levels were statistically significantly higher in animals dosed with α-solanine alone, as compared to controls.

Acetylcholinesterase and Buturylcholinesterase. There was no significant difference in AChE or BuChE activity in plasma or brain between groups (data not shown).

LC-MS Analysis of the Tissues and Fluids. LC-MS analysis of all plasma samples yielded no trace of α-chaconine, α-solanine, or their metabolites including the aglycone. The metabolomics approach did however corroborate the finding of the clinical chemistry with increases in creatine in the animals given α-solanine or α-chaconine alone or the high combined dose, as compared to controls. In addition, the glucose levels in the animals fed α-solanine alone were significantly greater than those seen in the controls. Circulating cholesterol levels were not significantly different between the treated groups and the control.

Metabolomics analysis of the urine showed no traces of any putative GA-derived sulfonation and glucuronide metabolites.

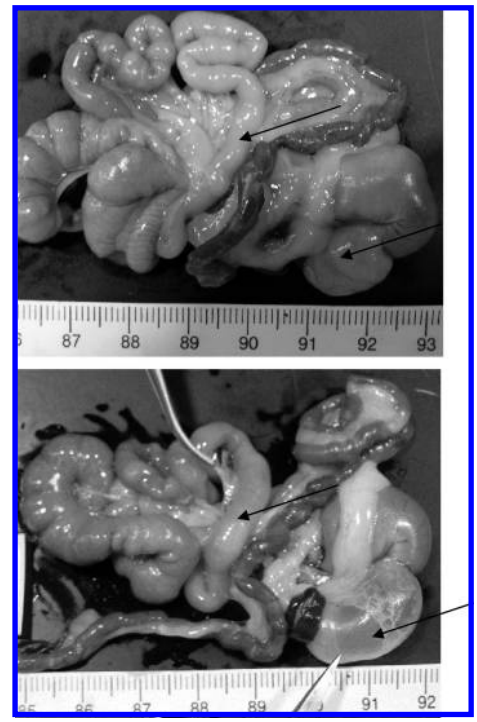

Figure 1. Gastrointestinal tract of the Syrian Golden hamster. The top picture shows the gastrointestinal tract from a control animal. The bottom picture shows how the small intestine and stomach were dilated and fluid filled in an animal given GA. Arrows indicate the small intestine and stomach when dilated and air- and fluid-filled in the dosed animal as compared to corresponding areas in the control animal.

However, in the urine samples from the chaconine group, the β_1, β_2, and γ derivatives of α-chaconine were evident (**Figures 2** and **3**). The hydrolysis products were below the limits of quantification of the analytical method (15 μg mL^{-1}). The single ion MS monitoring chromatograms for β_1, β_2, and γ derivatives

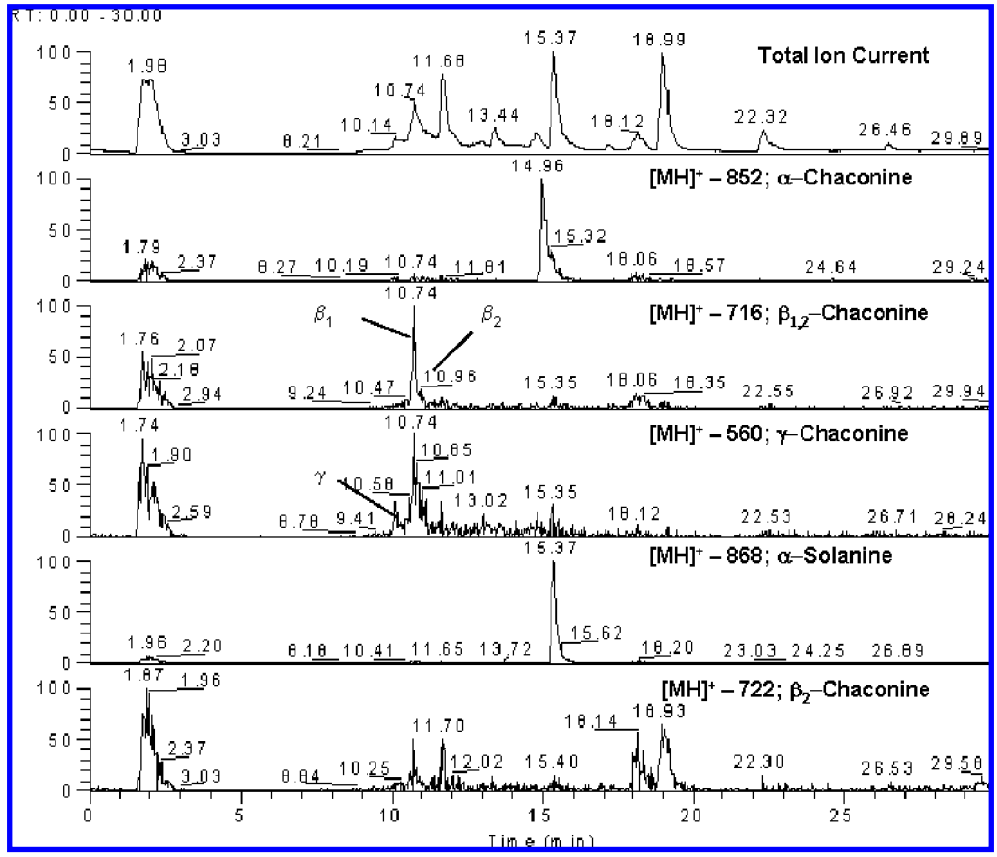

Figure 2. LC-MS of hamster urine following feeding with α-chaconine and α -solanine showing evidence of GA metabolism in the form of the glycolytic products β-1,2- and γ-chaconine. No glycolytic derivatives of α-solanine were detected.

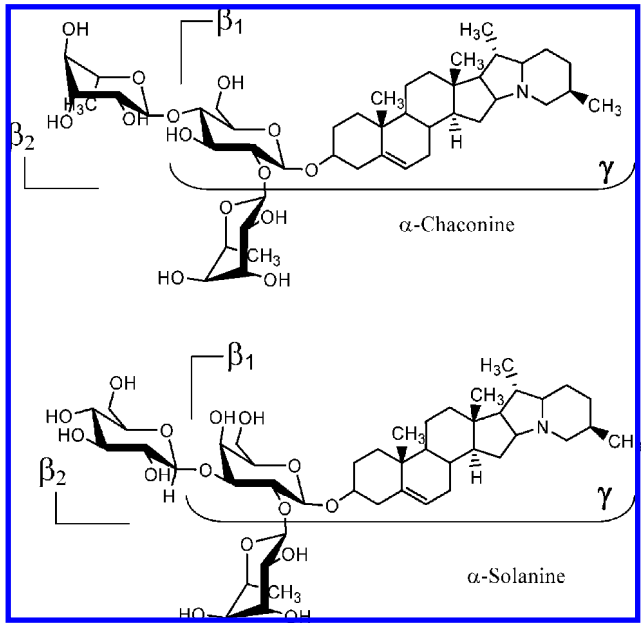

Figure 3. α-Chaconine (solanidine bis-α-L-rhamnopyranosyl-α-D-glucopyranose), α-solanine (solanidine α-L-rhamnopyranosyl-α-D-glucopyranosyl-α-galactopyranose), and their glycolytic metabolites β_1, β_2, and γ (cumulative losses for both β_1 and β_2).

of α-solanine were much less convincing and approaching the limits of detection but did suggest that traces of these ($\beta > \gamma$) were present in the urine (**Figure 2**). However, the presence of unmetabolized α-solanine in the urine was definitely evident and detectable.

Analysis of the tissues (intestine and stomach content and liver) showed that for GA treatments containing α-chaconine there was a presence of this GA in the liver tissues only (~ng levels). No trace of either the β_1, β_2, and γ derivatives of α-chaconine metabolites or α-solanine and its β_1, β_2, and γ derivatives were found in any of the tissues.

Extensive metabolic profiling was performed on tissues, biofluids and wastes. The resulting data were analyzed by PCA; no segregation based on either GA type or dosage was observed.

DISCUSSION

The objective of the study was to examine toxic effects of high doses of α-solanine and α-chaconine, alone or combined, administered orally by gavage to the Syrian Golden hamster daily for 5 days. Previous studies have demonstrated that 50 mg of α-solanine and α-chaconine in a 1:1 ratio, given per oral, was not lethal to hamsters (*24*). The results generated by Phillips et al. (*24*) and by our study indicate that the lethal dose for the Syrian Golden hamster of the GAs lies above 50 and below 100 mg (kg BW)$^{-1}$. According to the literature, toxicity of GAs may apply through two main mechanisms, either by inhibition of the activity of AChE and BuChE enzymes or by disruption of cell membranes in the gastrointestinal tract (*7, 13, 25*).

There were no detectable effects of GAs on AChE or BuChE activity in brain or plasma in the hamsters in this study. Several previous studies (*7, 11, 13, 14, 16*) demonstrated inhibition of AChE activity both in vivo (brain) and in vitro. However, the results from these in vivo studies are rather inconsistent concerning the effects of GA on esterase activity. One study reported an inhibition of brain AChE activity as compared to controls in hamsters gavaged either 400 or 500 mg of potato sprout material. In the same study, administration of 300 mg of potato sprout material to hamsters resulted in an increase in brain AChE activity (*14*).

Male Sprague−Dawley rats were given doses of α-solanine by gavage at 250 mg (kg BW)$^{-1}$ or i.p. (intraperitoneal) at 20 mg (kg BW)$^{-1}$. In the orally dosed animals, the serum cholinesterase activity was decreased, but the difference was not statistically significant. With the i.p.-dosed animals, a statistically significant decrease of 27% in serum cholinesterase activity was observed (*26*). However, care needs to be taken in comparisons between results from the testing of materials from potatoes and the individual pure GAs since the studies wherein potato sprout material has been used can be heavily biased toward the effects of α-chaconine since the biosynthesis of this specific GA can predominate in the sprouts (*4*), with α-solanine:α-chaconine ratios varying from ∼1:1 to 1:1580, respectively, depending upon the variety and condition (damage or greening) of the tuber (*4*).

In vitro studies have shown an inhibition of AChE and BuChE activity induced by α-solanine and α-chaconine (*16*). The lack of effect in the present study and the equivocal effects in the literature could be interpreted as a consequence of the considerable differences in the metabolism of compounds in vivo and in vitro.

As the present in vivo study does not show any effect of GAs on AChE activity, the lethality caused by the GAs may be attributed to the cytotoxic properties of α-solanine and α-chaconine. Macroscopic observations revealed that animals, dosed with α-solanine alone or in combination with α-chaconine in five consecutive days, had fluid-filled and distended intestines and stomach. These effects may have been a primary or secondary effect from local membrane disruption in the gastrointestinal tract caused by α-solanine. No consistent histological changes in intestine, stomach, or other organs, directly related to GA administration, were observed.

Statistically significant increased water consumption was observed for groups dosed with 100 mg of α-solanine alone or 100 mg of α-solanine and α-chaconine combined. Likewise, levels of sodium, glucose, and creatine were statistically significantly higher in dosed groups as compared to controls. This could indicate that the fluid balance in the dosed animals was somehow affected by the administration of GAs, and the results comply with the distended and fluid-filled intestines observed for animals of the same dose groups.

Levels of rbc, hct, and hgb were statistically significantly higher in treatment groups as compared to controls. This may also indicate that hamsters administered GAs suffered from some degree of dehydration in spite of increased water intake.

It has previously been demonstrated that both α-chaconine and α-solanine depolarize membranes in frog embryos (*25*). Alterations in the membrane potential can be caused by GA-induced effects on mechanisms involved in the active epithelial transport of ions. Disruption of carriers and ionic pumps may affect fluid and ion balance, cause membrane depolarization, and ultimately result in cell lysis. Another study showed that the short-circuit current, which is a direct measure of sodium transport across the cell membrane, was reduced by α-chaconine and α-solanine in frog skin (*27*). It is therefore likely that α-solanine and possibly α-chaconine could have induced cell disruption in dosed animals in the present study, by affecting the active ion transport across the cell epithelia, causing loss of fluid to the intestinal tract and elevated levels of creatine, sodium, glucose, rbc, hct, and hgb in the blood plasma. The higher level of creatine may actually depict releases from damaged muscular cells. Whether changes of blood chemistry and hematology induced by GA doses were reversible in the present study were not examined.

Statistically significant decreased liver weights and decreased levels of albumin, together with lower levels of alkaline phosphatase in dosed animals point to an effect of GA on the liver. Metabolism of GAs may have induced this effect. Indeed, LC-MS analysis showed that there was definite evidence for α-chaconine presence in the liver of animals treated with this GA, but no α-solanine presence was seen. This is in agreement with previous GA studies in mice (*28*) and hamsters (*11*) that suggested that the relative durability and/or presence of α-chaconine in the liver, which was characterized by two accumulation maxima, the second smaller, was due to enterohepatic recycling (*29*). Nevertheless, the strongest effects on the liver parameters are seen in the groups getting the highest dosages of solanine (**Table 5**). Friedman et al. (*30*) showed that three aglycone forms of steroidal GAs, solanidine, solasodine, and tomatidine, induced an increase in liver weight in nonpregnant and pregnant mice as opposed to the reduced liver weights induced by α-solanine and α-chaconine in the present study. Caldwell et al. (*31*) showed that hepatic ornithine decarboxylase (ODC) activity in rat livers was increased up to 8 h after i.p. injections of α-chaconine and to a lesser degree α-solanine. The activity of ODC was not measured in the present study.

Interestingly, there was no evidence in the liver of GA metabolism in the form of glycolytic degradation to β_1-, β_2-, and γ-chaconine, the aglycone solanidine, or the products of the common hepatic detoxification processes such as glucuronidation (*32*) and/or sulfonation (*33*). In addition, neither the potential glucuronidation nor the sulfonation products were detected in the urine. However, there was clear evidence from LC-metabolomic analysis of α-chaconine glycolysis with the corresponding β_1, β_2, and γ derivatives present in the urine. This action is unlikely to be purely chemical rather than enzymatic; otherwise, the analogous solanine derivatives would have also been reported. Interestingly, this glycolytic action could well be a mechanism to reduce deleterious toxic effects since studies by Rayburn et al. (*34*) found that the toxicity of α-chaconine, as measured by mortality and malformation levels in a frog embryo teratogenicity assays (*Xenopus*), was significantly greater than β_1-, β_2-, and γ-chaconine and that toxicity and teratogenicity were reduced each time a saccharide residue was removed ($\alpha > \beta_1 > \beta_2 > \gamma$). The hamster is able to metabolize chaconine the same way (**Figure 2**), thereby possibly detoxifying chaconine more effectively than solanine, which is excreted unchanged in the urine (**Figure 2**). The inability of the hamster to immediately metabolize and detoxify the solanine may be the reason for the solanine to be more toxic to the hamster than chaconine.

Synergistic effects on cell membrane disruption from α-solanine and α-chaconine have been described in a number of in vitro studies (*35, 36, 24*). Data from the present study do not point to a synergistic effect of GAs combined but indicate that α-solanine is more acute toxic than α-chaconine. In accordance Friedman et al. (*37*), they showed that mixtures of α-chaconine and α-solanine exhibited synergistic toxic effects on HepG2 liver cells with proportions 0.1:0.9, 0.3:0.7, and 0.5:0.5 (chaconine to solanine), whereas a proportion of 0.7:0.3 pointed to an additive effect. In the present study, mixture solutions were applied as α-solanine to α-chaconine, 1:2.5. This mixture corresponds to a proportion of α-chaconine to α-solanine of approximately 0.7:0.3.

Roddick (*38*) showed that GAs form strong complexes in vitro with constituents of the food, such as cholesterol. The cytotoxic properties of GAs may be related to the ability of GAs to bind cholesterol-containing cell membranes in the

gastrointestinal tract. In the light of this, future studies may examine GAs incorporated into a solid diet rather than administering GAs by gavage.

In the present study, doses from 100 mg α-solanine or α-chaconine (kg BW)$^{-1}$ alone or 75 or 100 mg α-solanine and α-chaconine (kg BW)$^{-1}$ combined given daily in 5 days were lethal to at least one of four dosed Syrian Golden hamsters within 4−5 days. In humans, doses of 3−6 mg GAs (kg BW day)$^{-1}$ are potentially lethal according to the literature (*7*). The dose level, necessary to induce a systemic, toxic effect in the Syrian hamster, signifies that humans are far more sensitive to the effects of GAs as compared to the hamster. Although the present study is not an acute toxicity study in the classical sense since it is dosing the hamster for five consecutive days, it is clear that the solanine:chaconine (1:2.5) level of 75 mg (kg BW day)$^{-1}$ is certainly not the no-effect level (NEL) for toxicity of the GAs in the Golden Syrian hamster, since the liver weight is significantly decreased. Further studies are needed to identify a "true" NEL in hamster to create the scientific basis for a more formal risk assessment.

ABBREVIATIONS USED

GA, glycoalkaloid; BW, body weight; AChE, acetyl cholinesterase; BuChE, butyryl cholinesterase; wbc, total leukocyte count; rbc, erythrocyte count; plt, platelets; hgb, hemoglobin; hct, hematocrit; mcv, mean cell volume; mch, mean corpuscular hemoglobin; mchc, mean corpuscular hemoglobin concentration; PDA, photodiode array detector; ESI-MS, electrospray ionization−mass spectrometry; i.p., intraperitoneal; NEL, no-effect level; ODC, hepatic ornithine decarboxylase.

ACKNOWLEDGMENT

The study is part of the sixth framework programme EU project Quantitative Risk Assessment Strategies for Novel Foods: NOFORISK, designed to develop and validate new methodologies for assessing the safety of a "second generation" novel food using a gene-modified potato as a model. We acknowledge the technical assistance of Ditte Sørensen, Merete Lykkegaard, Karen Roswall, Anne Ørngren, and animal technicians, Joan Frandsen and Annette Landin. We thank Kirsten Pilegaard and Ole Vang for their recommendations in the process of writing.

LITERATURE CITED

(1) Friedman, M.; McDonald, G. M. Potato glycoalkaloids: chemistry, analysis, safety and plant physiology. *Crit. Rev. Plant Sci.* **1997**, *16*, 55–132.

(2) Sotelo, A.; Serrano, B. High-performance liquid chromatographic determination of the glycoalkaloids α-solanine and α-chaconine in 12 commercial varieties of Mexican potato. *J. Agric. Food Chem.* **2000**, *48*, 2472–5.

(3) Machado, R. M. D.; Toledo, M. C. F.; Garcia, L. C. Effect of light and temperature on the formation of glycoalkaloids in potato tubers. *Food Control* **2007**, *18*, 503–508.

(4) Şengul, M.; Keleş, F.; Keleş, M. S. The effect of storage conditions (temperature, light, time) and variety on the glycoalkaloid content of potato tubers and sprouts. *Food Control* **2004**, *15*, 281–286.

(5) Chaube, S.; Swinyard, C. A. Teratological and toxicological studies of alkaloidal and phenolic compounds from *Solanum tuberosum* L. *Toxicol. Appl. Pharmacol.* **1976**, *36*, 227–237.

(6) Smith, D. B.; Roddick, J. G.; Jones, J. L. Potato glycoalkaloids: Some unanswered questions. *Trends Food Sci. Technol.* **1996**, *7*, 126–131.

(7) Morris, S. C.; Lee, T. H. The toxicity and teratogenicity of Solanaceae glycoalkaloids, particularly those of the potato (*Solanum tuberosum*): A review. *Food Technol. Aust.* **1984**, *36*, 118–124.

(8) Hopkins, J. The glycoalkaloids: Naturally of interest (but a hot potato?). *Food Chem. Toxicol.* **1995**, *33*, 323–328.

(9) Mensinga, T. T.; Sips, A. J.; Rompelberg, C. J.; van Twillert, K.; Meulenbelt, J.; van den Top, H. J.; van Egmond, H. P. Potato glycoalkaloids and adverse effects in humans: An ascending dose study. *Regul. Toxicol. Pharmacol.* **2005**, *41*, 66–72.

(10) Patel, B.; Schutte, R.; Sporns, P.; Doyle, J.; Jewel, L.; Fedorak, R. N. Potato glycoalkaloids adversely affect intestinal permeability and aggravate inflammatory bowel disease. *Inflamm. Bowel Dis.* **2002**, *8*, 340–346.

(11) Alozie, S. O.; Sharma, R. P.; Salunkhe, D. K. Physiological disposition, subcellular distribution and tissue binding of α-chaconine (^{3}H). *J. Food Saf.* **1978**, *1*, 257–273.

(12) Alozie, S. O.; Sharma, R. P.; Salunkhe, D. K. Excretion of alpha-chaconine-^{3}H, and steroidal glycoalkaloid from *Solanum tuberosum* L. and its metabolites in hamsters. *Pharmacol. Res. Commun.* **1979**, *11*, 483–490.

(13) Baker, D. C.; Keeler, R. F.; Garfield, W. P. Mechanism of death in Syrian hamsters gavaged potato sprout material. *Toxicol. Pathol.* **1988**, *16*, 333–339.

(14) Groen, K. Bioavailabjlity and disposition of α-solanine in rat and hamster. *Xenobiotica* **1993**, *93*, 995–1005.

(15) McGehee, D. S.; Krasowski, M. D.; Fung, D.; Wilson, B.; Gronert, G. A.; Moss, J. Cholinesterase inhibition by potato glycoalkaloids slows *Mivacurium* metabolism. *Anesthesiology* **2000**, *93*, 510–519.

(16) Renwick, J. H.; Claringbold, W. D.; Earthy, M. E.; Few, J. D.; McLean, A. C. Neural-tube defects produced in Syrian hamsters by potato glycoalkaloids. *Teratology* **1984**, *30*, 371–381.

(17) Bushway, R. J.; Savage, S. A.; Ferguson, B. S. Inhibition of acetyl cholinesterase by solanaceous glycoalkaloids and alkaloids. *Am. Potato J.* **1987**, *62*, 301–306.

(18) Kluge, W. H.; Kluge, H. H.; Bauer, H. I.; Pietsch, S.; Anders, J.; Venbrocks, R. A. Acetylcholinesterase assay for cerebrospinal fluid using bupivacaine to inhibit butyrylcholinesterase. *BMC Biochem.* **2001**, *2*, 17–25.

(19) Dobson, G.; Shepherd, T.; Marshall, R.; Verrall, S. R.; Conner, S.; Griffiths, D. W.; McNicol, J. W.; Davies, H. V.; Stewart, D. Application of metabolite and flavour volatile profiling to studies of biodiversity in *Solanum* species. Proceedings of the Third International Congress on Plant Metabolomics, Ames, Iowa, June 2004.

(20) Shepherd, T.; Dobson, G.; Verrall, S. R.; Conner, S.; Griffiths, D. W.; McNicol, J. W.; Davies, H. V.; Stewart, D. GC-MS potato metabolomics: What are the limiting factors. **2007**, *3*, 475−488.

(21) Lee, K. R.; Kozukue, N.; Han, J. S.; Park, J. H.; Chang, E. Y.; Baek, E. J.; Chang, J. S.; Friedman, M. Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells. *J. Agric. Food Chem.* **2004**, *52*, 2832–2839.

(22) Friedman, M.; McDonald, G. M. Acid-catalyzed partial hydrolysis of carbohydrate groups of the potato glycoalkaloid a-chaconine in alcoholic solutions. *J. Agric. Food Chem.* **1995**, *43*, 1501–1506.

(23) Friedman, M.; McDonald, G. M.; Haddon, W. F. Kinetics of acid-catalyzed hydrolysis of carbohydrate groups of potato glycoalkaloids α-solanine and α-chaconine. *J. Agric. Food Chem.* **1993**, *41*, 1397–1406, 1993.

(24) Phillips, B. J.; Hughes, J. A.; Phillips, J. C.; Walters, D. G.; Anderson, D.; Tahourdin, C. S. A study of the toxic hazard that might be associated with the consumption of green potato tops. *Food Chem. Toxicol.* **1996**, *34*, 439–448.

(25) Blankemeyer, J. T.; Stringer, B. K.; Rayburn, J. R.; Bantle, J. A.; Friedman, M. Effect of potato glycoalkaloids, α-chaconine and α-solanine on membrane potential of frog embryos. *J. Agric. Food Chem.* **1992**, *40*, 2022–2025.

(26) Dalvi, R. R. Comparative assessment of the effect of solanine administered orally and intraperitoneally on hepatic dysfunction in male rats. *Jpn. J. Vet. Sci.* **1985**, *47*, 657–659.

(27) Blankemeyer, J. Tr.; Atherton, R.; Friedman, M. Effect of potato glycoalkaloids α-chaconine and α-solanine on sodium

active transport in frog skin. J. Agric. Food Chem. **1995**, *43*, 636–639.
(28) Sharma, R. P.; Taylor, M. J.; Bourcier, D. R. Subcellular distribution of alpha-chaconine in mouse hepatocytes. Drug Chem. Toxicol. **1983**, *6*, 219–234.
(29) Kuiper-Goodman, T.; Nawrot, P. S. Solanine and chaconine. *WHO Food Additives*; Series 30; WHO: Geneva, Switzerland., 1993.
(30) Friedman, M.; Henika, P. R.; Mackey, B. E. The effect of feeding solanine, solasodine and tomatodine to non-pregnant and pregnant mice. Food Chem. Toxicol. **2003**, *41*, 61–71.
(31) Caldwell, K. A.; Grojean, U. K.; Henika, P. R.; Friedman, M. Hepatic ornithine secarboxylase induction by potato glycoalkaloids in rats. *Phytochemistry* **1979**, 1467–1470.
(32) Ritter, J. K. Roles of glucuronidation and UDP-glucuronosyltransferases in xenobiotic bioactivation reactions. Chem.-Biol. Interact. **2000**, *129*, 171–193.
(33) Strott, C. A. Sulfonation and molecular action. Endocr. Rev. **2002**, *23*, 703–732.
(34) Rayburn, J. R.; Bantle, J. A.; Friedman, M. Role of carbohydrate side chains of potato glycoalkaloids in developmental toxicity. J. Agric. Food Chem. **1994**, *42*, 1511–1515.
(35) Roddick, J. G.; Rijnenberg, A. L.; Osman, S. F. Synergistic interaction between potato glycoalkaloids α-solanine and α-chaconine in relation to lysis of phospholipids/sterol liposomes. Phytochemistry **1987**, *26*, 1325–1328.
(36) Roddick, J. G.; Rijnenberg, A. L.; Osman, S. F. Synergistic interaction between potato glycoalkaloids α-solanine and α-chaconine in relation to destabilization of cell membranes: ecological implications. J. Chem. Ecol. **1988**, *14*, 889–902.
(37) Friedman, M.; Lee, K.-R.; Kim, H.-J.; Lee, I.-S.; Kozikue, N. Anticarcinogenic effects of glycoalkaloids from potatoes against human cervical, liver lymphoma and stomach cancer cells. J. Agric. Food Chem. **2005**, *53*, 6162–6169.
(38) Roddick, J. G. Complex formation between solanaceous steroidal glycoalkaloids and free sterols in vitro. Plant Physiol. Biochem. **1979**, *18* (9), 1467–1470.

Received for review May 6, 2008. Revised manuscript received July 4, 2008. Accepted July 8, 2008.

JF8012794