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**A pilot study on the pharmacokinetics of  
potato glycoalkaloids in healthy volunteers.**

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## Abstract

To improve risk assessment of the potato glycoalkaloids (GAs)  $\alpha$ -solanine and  $\alpha$ -chaconine, a study on pharmacokinetics of orally administered GAs in humans is planned to be carried out at the National Institute of Public Health and the Environment (RIVM). The limited information on toxicity in relation to GA dose in humans urged the need to first perform a dose finding study (pilot study) to find an optimal blood sampling scheme and an optimal GA dose for a subsequent study on pharmacokinetics of GAs.

In the pilot study, subjects received either a solution containing  $\alpha$ -solanine and  $\alpha$ -chaconine (GA dose: 0.20, 0.30, 0.50 or 0.70 mg/kg body weight) or a portion of mashed potatoes containing known amounts of  $\alpha$ -solanine and  $\alpha$ -chaconine (GA dose: 0.80, 0.95, 1.10 and 1.25 mg/kg body weight). After each dose administration, pharmacokinetics and possible adverse/toxic effects were evaluated. From the results of the pilot study, an optimal blood sampling scheme could be obtained. Furthermore an optimal GA dose for mashed potatoes was found: a GA dose  $\geq 0.95$  and  $\leq 1.00$  mg/kg body weight. For the test solution, it was expected that a GA dose of 0.90 mg/kg body weight would be appropriate for the full study. The performance of the full study, using these dose levels, will give more insight (at least for  $\alpha$ -chaconine) in the kinetics of potato GAs in humans.

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## Samenvatting

Aardappelen bevatten de glycoalkaloïden (GAs)  $\alpha$ -solanine and  $\alpha$ -chaconine. Van deze stoffen is bekend dat ze toxisch zijn voor de mens en andere species. In het algemeen worden gehaltes tot 200 mg GAs per kg aardappelen als veilig beschouwd voor de mens. Deze grens is echter gebaseerd op een summiere onderbouwing daar de benodigde informatie over de toxiciteit en farmacokinetiek van GAs ontbreken. Om de risicoschatting te verbeteren zal er bij het RIVM een studie naar de farmacokinetiek van oraal toegediende GAs bij de mens uitgevoerd gaan worden. Met de verkregen farmacokinetiekdata zullen toxiciteitsstudies bij proefdieren geïnterpreteerd kunnen worden en zullen beleidsmakers een weloverwogen beslissing kunnen nemen of de huidige grens van 200 mg GAs per kg aardappelen voldoet of aangepast moet worden. Voordat de geplande farmacokinetiekstudie uitgevoerd kan worden, was het noodzakelijk om een voorstudie uit te voeren. Dit werd veroorzaakt door de beperkte informatie over de toxiciteit van GAs bij de mens in relatie tot de dosis. De doelstelling van de voorstudie was het vinden van 1) een geschikt bloedafnameschema en 2) een geschikte GA-dosering voor het bepalen van de farmacokinetiek van GAs in de hoofdstudie. Het bloedafnameschema is geschikt als dit resulteert in serum concentratie-tijd curves voor  $\alpha$ -solanine en  $\alpha$ -chaconine, waarmee de farmacokinetische parameters goed berekend kunnen worden. De GA-dosering is geschikt als deze enerzijds meetbare GA-concentraties in het serum oplevert en anderzijds geen toxische effecten veroorzaakt. In dit rapport worden de opzet en verkregen resultaten van de voorstudie beschreven.

In de voorstudie kregen proefpersonen één GA-dosering in de vorm van een drankje toegediend (toegediende GA-doseringen: 0,20, 0,30, 0,50 or 0,70 mg/kg lichaamsgewicht; GA bestond uit 50%  $\alpha$ -solanine and 50%  $\alpha$ -chaconine) of ze kregen één portie aardappelpuree te eten, die een bekende hoeveelheid  $\alpha$ -solanine en  $\alpha$ -chaconine bevatte (toegediende GA-doseringen: 0,80, 0,95, 1,10 and 1,25 mg/kg lichaamsgewicht; GA bestond uit 51%  $\alpha$ -solanine and 49%  $\alpha$ -chaconine). Na elke toediening werd op een aantal tijdstippen bloed afgenomen om het verloop van de  $\alpha$ -solanine- and  $\alpha$ -chaconineconcentratie in het serum te kunnen volgen in de tijd. De concentratie-tijd curves werden vervolgens geanalyseerd om zo de farmacokinetische parameters van  $\alpha$ -solanine and  $\alpha$ -chaconine ( $C_{\max}$ ,  $t_{\max}$ ,  $t_{1/2}$ ,  $AUC_{0-\infty}$ , en  $F_{\text{rel}}$ ) te kunnen bepalen. Tevens werden eventueel waargenomen toxische effecten geëvalueerd na elke toediening. Met behulp van de resultaten van de voorstudie, kon een geschikt bloedafnameschema voor beide toedieningsvormen worden gevonden. Als GA-dosering in aardappelpuree, bleek een GA-dosering  $\geq 0,95$  and  $\leq 1,00$  mg/kg lichaamsgewicht het meest geschikt. Bij deze doseringsrange kan de farmacokinetiek van  $\alpha$ -chaconine goed bepaald worden; voor  $\alpha$ -solanine kunnen bij deze dosering in ieder geval  $C_{\max}$  and  $t_{\max}$  goed bepaald worden. Afhankelijk van de verdere optimalisatie van de analysemethode in serum kunnen ook de overige parameters bepaald worden voor  $\alpha$ -solanine.

De onderzochte GA-doseringsrange voor het drankje leverde geen geschikte GA-dosering op: de GA-concentraties in het serum konden niet lang genoeg gemeten worden. Gebaseerd op de resultaten van de voorstudie wordt verwacht dat een GA-dosering van 0,90 mg/kg

lichaamsgewicht geschikt is om de farmacokinetiek van  $\alpha$ -solanine en  $\alpha$ -chaconine te bestuderen zonder dat daarbij toxische effecten optreden. Naar verwachting kan de farmacokinetiek van  $\alpha$ -chaconine goed bepaald worden bij deze dosering. Ook hier geldt voor  $\alpha$ -solanine dat bij deze dosering in ieder geval  $C_{\max}$  and  $t_{\max}$  goed bepaald kunnen worden. Afhankelijk van de verdere optimalisatie van de analysemethode in serum kunnen waarschijnlijk ook de overige farmacokinetische parameters bepaald worden voor  $\alpha$ -solanine. Hogere doseringen worden niet aanbevolen omdat toxische effecten dan niet kunnen worden uitgesloten.

Het rapport eindigt met een aantal aanvullende aanbevelingen voor de hoofdstudie en met een aantal belangrijke conclusies uit de voorstudie.

## Summary

Potatoes contain the glycoalkaloids (GAs)  $\alpha$ -solanine and  $\alpha$ -chaconine which are known to be toxic to humans and other species. Generally, 200 mg GAs per kg potatoes is accepted as safe level in humans. This level is based on limited evidence since proper information on GA toxicity and pharmacokinetics in humans are lacking. To improve risk assessment of GAs, a study on pharmacokinetics of orally administered GAs in humans is planned to be carried out at the National Institute of Public Health and the Environment (RIVM). With the gathered information on pharmacokinetics, data from toxicity studies in animals can be interpreted. This will enable health policy makers to make a conscious decision whether or not the present recommended limit of 200 mg GAs per kg potatoes needs adaptation.

Before the study on pharmacokinetics can be performed, a pilot study was required. The limited information on toxicity in relation to GA dose in humans necessitated a dose finding study (pilot study) to find 1) an optimal blood sampling scheme and 2) an optimal GA dose for a subsequent study on pharmacokinetics of GAs. The blood sampling scheme should result in serum concentration time curves from which adequate pharmacokinetic parameters can be obtained. The GA dose should be high enough to result in measurable serum GA concentrations but low enough to avoid toxic effects.

The design and results of the pilot study on pharmacokinetics of GAs are presented in the current report. In the pilot study, each subject received either a solution containing  $\alpha$ -solanine and  $\alpha$ -chaconine (GA dose: 0.20, 0.30, 0.50 or 0.70 mg/kg body weight; GA consisted of 50%  $\alpha$ -solanine and 50%  $\alpha$ -chaconine) or a portion of mashed potatoes containing known amounts of  $\alpha$ -solanine and  $\alpha$ -chaconine (GA dose: 0.80, 0.95, 1.10 and 1.25 mg/kg body weight; GA consisted of 51%  $\alpha$ -solanine and 49%  $\alpha$ -chaconine). After each administration, frequent blood samplings were performed to obtain serum concentration time curves of  $\alpha$ -solanine and  $\alpha$ -chaconine. These curves were subsequently analysed to determine pharmacokinetics of  $\alpha$ -solanine and  $\alpha$ -chaconine ( $C_{\max}$ ,  $t_{\max}$ ,  $t_{1/2}$ ,  $AUC_{0-\infty}$ , and  $F_{\text{rel}}$ ). In addition, possible adverse/toxic effects were evaluated after each dose administration.

An optimal blood sampling scheme to study pharmacokinetics of  $\alpha$ -solanine and  $\alpha$ -chaconine after administration of mashed potatoes and test solution, was obtained from the results of the pilot study.

To select the optimal GA dose for the full study, both adverse/toxic effects and the pharmacokinetic results of the pilot study were taken into account. From the results of the pilot study, it was concluded that for both  $\alpha$ -solanine and  $\alpha$ -chaconine, mashed potatoes containing a GA dose  $\geq 0.95$  and  $\leq 1.00$  mg/kg body weight is optimal for the full study. Within this dose range, pharmacokinetics of  $\alpha$ -chaconine can be determined adequately; regarding  $\alpha$ -solanine, at least  $C_{\max}$  and  $t_{\max}$  can be determined. Depending on the further optimisation of the analytical method, the other pharmacokinetic parameters of  $\alpha$ -solanine can be determined.

Regarding the test solution, it is concluded that the GA dose range studied in the pilot study did not include an optimal dose: serum GA concentrations could not be measured long



enough in time. Based on the results of the pilot study, it can be expected that a GA dose of 0.90 mg/kg body weight will be appropriate to study the basic pharmacokinetics of  $\alpha$ -chaconine and  $\alpha$ -solanine without the occurrence of adverse effects. It is expected that all pharmacokinetic parameters of  $\alpha$ -chaconine can be determined adequately at this dose. Regarding  $\alpha$ -solanine, at least  $C_{\max}$  and  $t_{\max}$  can be determined at this dose. Depending on the further optimisation of the analytical method in serum, the other pharmacokinetic parameters of  $\alpha$ -solanine can probably be determined. Higher doses are not recommended because adverse effects at these doses can not be excluded.

In addition to the blood sampling scheme and GA doses, some more recommendations for the full study are included in this report. The report ends with some important conclusions from the pilot study.

# 1. Introduction

## 1.1 Background

In 1820, Desfosses reported the discovery of a new organic base, isolated from berries of black nightshade (*Solanum nigrum*). One hundred milligrams of this compound, which he named solanée, caused considerable vomiting and unconsciousness when administered orally to a dog [1]. The *Solanaceae* family appeared to contain many plants important to man, both as agricultural crops like potato, tomato, bell pepper, and as weeds like nightshade [2]. A compound similar to solanée was found in potatoes and was named solanine [3]. The composition of solanine was finally revealed (130 years later) as a mixture of two glycosidic classes of glycoalkaloids (GAs), i.e. solanine and chaconine. Both classes have the same metabolite, the aglycone solanidine [4-6].

Figure 1.1 and table 1.1 describe some of the chemical characteristics of  $\alpha$ -solanine,  $\alpha$ -chaconine and solanidine.  $\alpha$ -Solanine and  $\alpha$ -chaconine are both triglycosides of the aglycone solanidine, a steroidal hexacyclic alkaloid derived from cholesterol. GAs are fairly heat-stable compounds; their melting points, at which some GAs may start decomposing, vary in general from 230 °C to 280 °C [7-9]. At these temperatures their aglycone, solanidine, shows no decomposition [10].

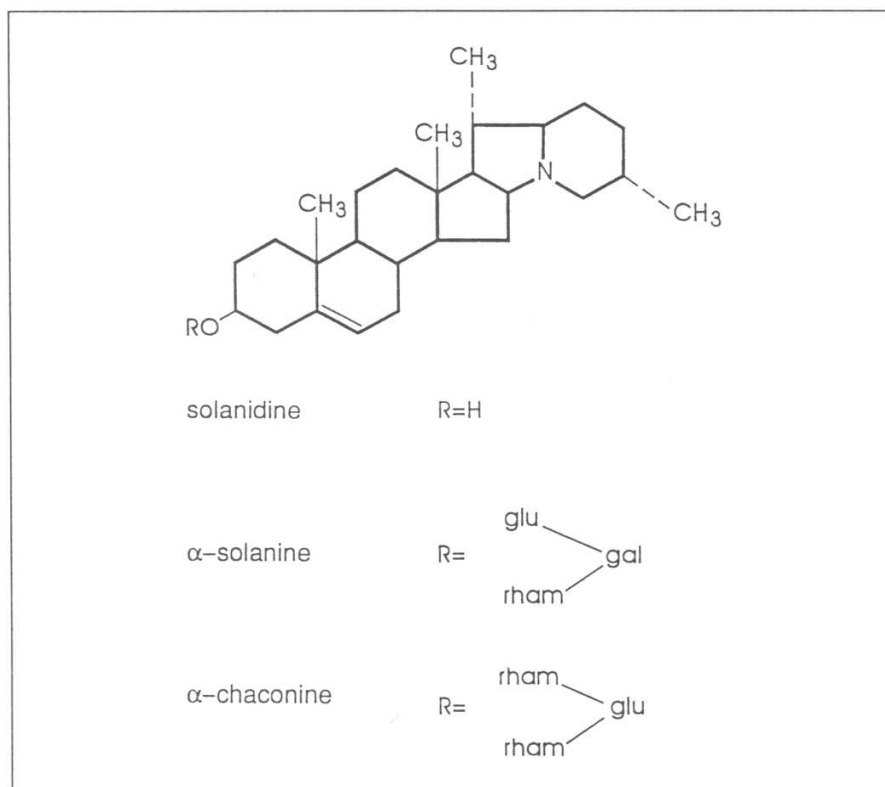


Figure 1.1 Structures of  $\alpha$ -solanine,  $\alpha$ -chaconine and their aglycone solanidine (glu= D-glucose; gal= D-galactose; rham= L-rhamnose)

*Table 1.1 Molecular formulae and weights of  $\alpha$ -solanine,  $\alpha$ -chaconine and their aglycone solanidine.*

Product	Molecular formula	Molecular weight
$\alpha$ -solanine	$C_{45}H_{73}NO_{15}$	868
$\alpha$ -chaconine	$C_{45}H_{73}NO_{14}$	852
solanidine	$C_{27}H_{43}NO$	398

The function of GAs was proposed to be protection of the plants against certain pests and diseases caused by insects and fungi. The GAs present in potatoes (*Solanum tuberosum*) are mainly  $\alpha$ -solanine and  $\alpha$ -chaconine. They are present in all organs of the plant, with the highest concentrations in regions of high metabolic activity such as young leaves and sprouts. Several factors during growth, harvesting and postharvest treatment, may lead to an increment of GA contents to high and toxic levels in the potato tuber, especially underneath the peel. Major factors causing this increment are genetic variation, growth conditions, light exposure, storage conditions and tuber injury [11,12]. It has been suggested that peeling removes 60 to 96% of these toxins. However, if potatoes contain high concentrations of GAs, they can be present throughout the flesh. Then, peeling may remove only 30 to 35% of the total amount present. [13-15].

## 1.2 Toxicity of potato glycoalkaloids

During the past two decades the attention for toxic effects of GAs has increased. Toxicity data derived from *in vitro* studies and animals studies, indicate that  $\alpha$ -chaconine is the most toxic alkaloid of the potato glycoalkaloids [16]. It exhibits strong lytic properties, is teratogenic, and inhibits acetylcholinesterase and butyrylcholinesterase [17].  $\alpha$ -Solanine exhibits less lytic properties, but likewise  $\alpha$ -chaconine it is a strong inhibitor of acetylcholinesterase and butyrylcholinesterase [17]. The aglycone solanidine is the least toxic GA [18-20]. Based on the present knowledge, humans seem to be more sensitive to GA intoxication than laboratory animals investigated. At lower doses, the toxicity of GAs in humans mainly concerns gastrointestinal disturbances like vomiting, diarrhoea and abdominal pain. The extent of the occurrence of these mild GA poisonings is unknown since the symptoms are common to many ailments with digestive discomfort [14,15,21,22]. At higher doses, however, the toxicity of GAs in humans concern more severe symptoms, with fever, rapid pulse, low blood pressure, rapid respiration, and neurological symptoms [23]. Even several cases of lethal poisoning due to GA exposure have been reported [24]. Furthermore, GAs may alter the pharmacokinetics of drugs metabolised by butyrylcholinesterase, like anaesthetic drugs (including succinylcholine, mivacurium, and cocaine), although conclusive experimental evidence on this is currently lacking [17].

Based on the data of 12 retrospective studies, Morris and Lee calculated the toxic dose of GAs in relation to body weight: toxic doses ranged from 2-5 mg/kg body weight, whereas lifethreatening doses ranged from 3-6 mg/kg body weight [24]. These conflicting ranges already emphasise the lack of information for a reliable deduction of a no observed adverse effect level (NO(A)EL) for humans. The above mentioned estimates are only partially informative, because the calculations were based on estimated values for GA contents in potatoes. Reported concentrations of GAs in potato run up to 800 mg/kg. Generally, 200 mg GAs per kg potatoes is accepted as the upper safe limit in humans, mainly because Bömer and Mattis concluded in 1924 that potatoes with contents of GAs exceeding 200 mg/kg are potentially hazardous to humans [25]. They had found that potatoes involved in poisoning showed contents of GAs exceeding 250 mg/kg fresh weight. Since then, many authors have interpreted this statement as ‘potatoes containing amounts of GAs below 200 mg/kg are safe for consumption’. However, surprisingly little information on the subacute and possible chronic toxicity of the GAs is available to support this statement [26]. In 1978, Ross et al. suggested that from the viewpoint of selecting cultivars for human consumption, the limit should be 60 to 70 mg/kg potatoes [27]. More recently Slanina et al. proposed to change the maximum tolerated dose to 100 mg GAs/kg unpeeled potato [28].

As indicated by the Joint Expert Committee on Food Additives and other international committees more adequate toxicological studies are needed [28-30]. The National Institute of Public Health and the Environment has already performed a subchronic toxicity study with  $\alpha$ -solanine in hamsters [31]. On the basis of this study the NOAEL is 225 mg  $\alpha$ -solanine/kg diet corresponding to 13.6 mg/kg body weight. If a safety evaluation was carried out by considering  $\alpha$ -solanine as food additive for daily food use, than probably a safety factor of at least 500 would have been applied because no chronic toxicity study is available. This would result in an acceptable daily intake (ADI) of 0-0.027 mg/kg body weight in humans. As  $\alpha$ -solanine is not a food additive but an inherent plant toxin present in an important food item, application of such an ADI would lead to an undesired low consumption of potato products from a nutritional point of view because potatoes provide nutrient and vitamins as bulk food in many countries.

### **1.3 Consumption exposure estimates of potato glycoalkaloids**

The ADI is not easily to be translated into complementary amounts of potatoes, since potatoes and potato products may contain variable amounts of GA. Assuming an average ‘daily’ intake for potatoes of 230 g [32] and an average daily intake of 10-20 g for crisps [based on average yearly consumption data (83 kg per person per year) from the Information Service for Food, Study Centre for Snacks & Sweets (Voorlichtingsbureau voor de Voeding, Studiecentrum Snacks & Zoetwaren), Zeist, the Netherlands], the following GA exposure can be calculated given various GA contents in potatoes.

*Table 1.2 Estimated single day GA exposures for different consumption situations and GA contents*

Consumption examples	GA-content 80 mg/kg potatoes	GA-content 200 mg/kg <sup>*</sup> potatoes	GA-content 800 mg/kg <sup>†</sup> potatoes
I: Potatoes 230 g • Adult 60 kg BW <sup>#</sup> • Child 30 kg BW	18.4 mg/subject 0.307 mg/kg BW 0.613 mg/kg BW	46.0 mg/subject 0.767 mg/kg BW 1.533 mg/kg BW	184.0 mg/subject 3.067 mg/kg BW 6.133 mg/kg BW
II: Potatoes 230 g + French fries 200 g • Adult 60 kg BW • Child 30 kg BW	34.4 mg/subject 0.573 mg/kg BW 1.147 mg/kg BW	86.0 mg/subject 1.433 mg/kg BW 2.867 mg/kg BW	344.0 mg/subject 5.733 mg/kg BW 11.467 mg/kg BW

\* The present allowable GA concentration in potatoes (upper safe limit).

† Reported concentrations of GA in potato run up to 800 mg/kg.

# BW= body weight

In fresh, unpeeled consumption potatoes the average GA content is 20-80 mg/kg [33]. These contents result in an average exposure of 0.307 mg/kg to 0.613 mg/kg body weight, a factor of 3 to 6 below the lower limit of the toxic dose range of 2 to 5 mg/kg body weight originally estimated by Morris and Lee [22]. These estimates, however, are based on average consumption amounts and average GA contents. Table 1.2, gives an indication of the consequences of exposure to higher GA contents and higher consumption amounts. The estimates show that the lower limit of toxic exposure levels (2 mg/kg body weight) might already be exceeded if subjects, especially children, on one day eat for example normal amounts of potatoes and French fries with GA contents of 200 mg/kg (third column). A similar consumption example would be the combination of 230 g potatoes and a family pack of crisps (200 g). The fourth column in table 1.2, with GA contents of 800 mg/kg, reveals outcomes in the lethal range.

The GA content of potatoes and potato products is influenced by the treatment of the potatoes/potato products before consumption. Peeling of potatoes (before boiling) removes 60 to 96% of the toxins. Gently removing the jacket after cooking does not seem to reduce the GA content in the potatoes. In industrial settings, techniques (peeling by means of steam) are applied in which it is likely that GA levels will remain high, mainly because of incomplete peeling (based on information from the Institute for Carbohydrate Studies (NIKO), Groningen, the Netherlands). If potatoes contain high concentrations of GAs, GAs are likely to be present throughout potato flesh. In this case, peeling before cooking may only remove 30 to 35% of the total amount present [13-15].

Furthermore, on a weight basis, GA levels can rise during the manufacturing of potato products, such as crisps and French fries. During frying of potato crisps it was found that the level of GAs increased due to dehydration. An increase from 40 to 150 mg/kg in crisps from peeled potatoes and from 40 to 250 mg/kg in crisps from unpeeled potatoes has been observed [34]. Sizer et al. showed that commercially purchased crisps revealed to have GA contents up to 720 mg/kg (reported in one study). Close examination of the crisps containing the highest levels revealed that 75-90% of the skin remained in the crisps, thus contributing to this high value [34].

Potatoes containing elevated levels of GAs (> 100 mg/kg) cause a bitter taste or burning sensation in the throat when consumed [27,35]. This has been suggested as indicative for levels of GAs in potatoes too high for safe consumption. However, certain methods of food preparation can mask the bitter taste [34]. It is conceivable that bitterness is easily unnoticed in crisps, probably because of taste masking by salt or oil. The bitterness may also vary with different ratios of  $\alpha$ -solanine and  $\alpha$ -chaconine. For instance  $\alpha$ -chaconine is much more bitter than  $\alpha$ -solanine. Moreover, individuals seem to vary in their perception of GA bitterness [36]. These factors and the many cases of GA poisoning show that the taste of potatoes is not a reliable indicator for GA levels too high for consumption.

To summarise, the consumption exposure estimates seem to indicate that public health is potentially at risk for  $\alpha$ -solanine and  $\alpha$ -chaconine intoxication. Therefore, more adequate human risk assessment data, like information on the pharmacokinetics of GAs should be provided.

## **1.4 Kinetics of potato glycoalkaloids in man**

Laboratory animals appear to be less sensitive to GA toxicity than man. In order to find out whether these differences are due to differences in pharmacokinetics (how much of the compound is absorbed, how long does it stay in the body, in which form does it stay in the body, by which route is the compound eliminated, etc.?) or in pharmacodynamics (what is the relation between the amount of compound at receptor site and the effects), pharmacokinetics of  $\alpha$ -solanine,  $\alpha$ -chaconine and solanidine need to be investigated in humans.

### **1.4.1 Absorption and bioavailability**

Most studies on potato GAs in man were not designed to study the pharmacokinetics of these compounds. For that reason no reliable data could be obtained for determining the fraction absorbed or for determining oral bioavailability of GAs. Only data, like the maximum serum concentration ( $C_{\max}$ ) and the time at which this concentration is achieved ( $t_{\max}$ ), which both can be regarded as indicative for absorption, were generated by Hellenäs et al. [37]. They performed a study in healthy volunteers in which blood serum levels of  $\alpha$ -solanine,  $\alpha$ -chaconine and solanidine were measured during a 25-h period following a single meal of mashed potatoes (equivalent to 1 mg GAs/kg body weight). Maximum concentrations of  $\alpha$ -solanine and  $\alpha$ -chaconine were achieved 5 and 6 hours postdose, respectively; whereas  $C_{\max}$  was 8 ng/ml for  $\alpha$ -solanine and 14 ng/ml for  $\alpha$ -chaconine.  $C_{\max}$  of the aglycone solanidine could not be determined accurately because the blood sampling scheme was not optimal for studying the pharmacokinetics of solanidine. Detectable amounts of solanidine appeared after 4-8 hours in all subjects. The highest solanidine values were found at 8 or 25 hours after the potato meal and varied among the subjects between 1 and 5 ng/ml.

### 1.4.2 Biotransformation and distribution

*Biotransformation:* Although only  $\alpha$ -solanine and  $\alpha$ -chaconine are normally present in significant amounts in the human diet, it is likely that they can be metabolised by hydrolysis of their side-chain glycoside:  $\alpha$ -solanine and  $\alpha$ -chaconine can be hydrolysed to their respective  $\beta$ - and  $\gamma$  forms. Subsequently, the  $\gamma$ -forms can be hydrolysed into the aglycone solanidine (see figure 1.2). In the study performed by Hellenäs et al. only the parent compounds ( $\alpha$ -forms) and the aglycone could be detected in HPLC-chromatograms of human serum, following oral administration of  $\alpha$ -solanine and  $\alpha$ -chaconine via mashed potatoes. There were no traces of the  $\beta$ - or  $\gamma$ -glycosides [37]. Solanidine may either be present in the potato tuber or may be formed in the human body (via intestinal or hepatic metabolism). It seems likely that solanidine is mainly formed in the human body, because the amount of solanidine present in potatoes is negligible [16]. Moreover, Hellenäs et al. [37] reported significant serum levels of solanidine following oral administration of GAs via mashed potatoes. Thus, besides the parental  $\alpha$ -solanine and  $\alpha$ -chaconine only the aglycone solanidine could be detected in human serum.

The site(s) at which hydrolysis take place is not clear. In the study mentioned above, mean serum total alkaloid concentrations were about 2.7 times the solanidine concentration, which, according to the authors, suggest considerable metabolism in man of  $\alpha$ -solanine and  $\alpha$ -chaconine through hydrolysis of the sugar residues. It was suggested that hydrolysis could take place in the acid medium of the stomach, or at in the gastrointestinal tract at the site of absorption [38]. However, no solid evidence was given for gastrointestinal metabolism of solanine into solanidine by the results of this study. Moreover, the study was performed in rats and due to interspecies differences it is not clear whether this hypothesis is also valid for the situation in humans.

*Distribution:* Claringbold et al. studied the kinetics and retention of intravenously administered tritiated solanidine in man [39], in which it was presumed that radioactivity represents solanidine or its metabolites. Within minutes of injection, the concentration of tritium in erythrocytes exceeded that in plasma. Erythrocytes were found to be a mobile reserve for solanidine, thereby delaying transfer of solanidine from vascular to extravascular compartments in which it is distributed. In histologically normal post-mortem livers from human subjects, solanidine was present in three of the five samples of human liver [39]. This suggests that solanidine accumulates in liver tissue. The literature does not make clear whether solanidine is also distributed to other organs or tissues.

### 1.4.3 Excretion

Terminal elimination half-lives for  $\alpha$ -solanine and  $\alpha$ -chaconine were calculated to be 10.7 and 19.1 h, respectively in a single oral dose study of Hellenäs [37]. However, it is not likely that these values represent terminal elimination half-lives, because the serum concentration-time curves suggest that the elimination phase had not been reached within this study duration. The data mentioned above therefore may underestimate the real terminal elimination half-life.

Claringbold et al. studied the kinetics and retention of intravenously administered tritiated solanidine in man [39]. Presuming that radioactivity represented solanidine or its metabolites,

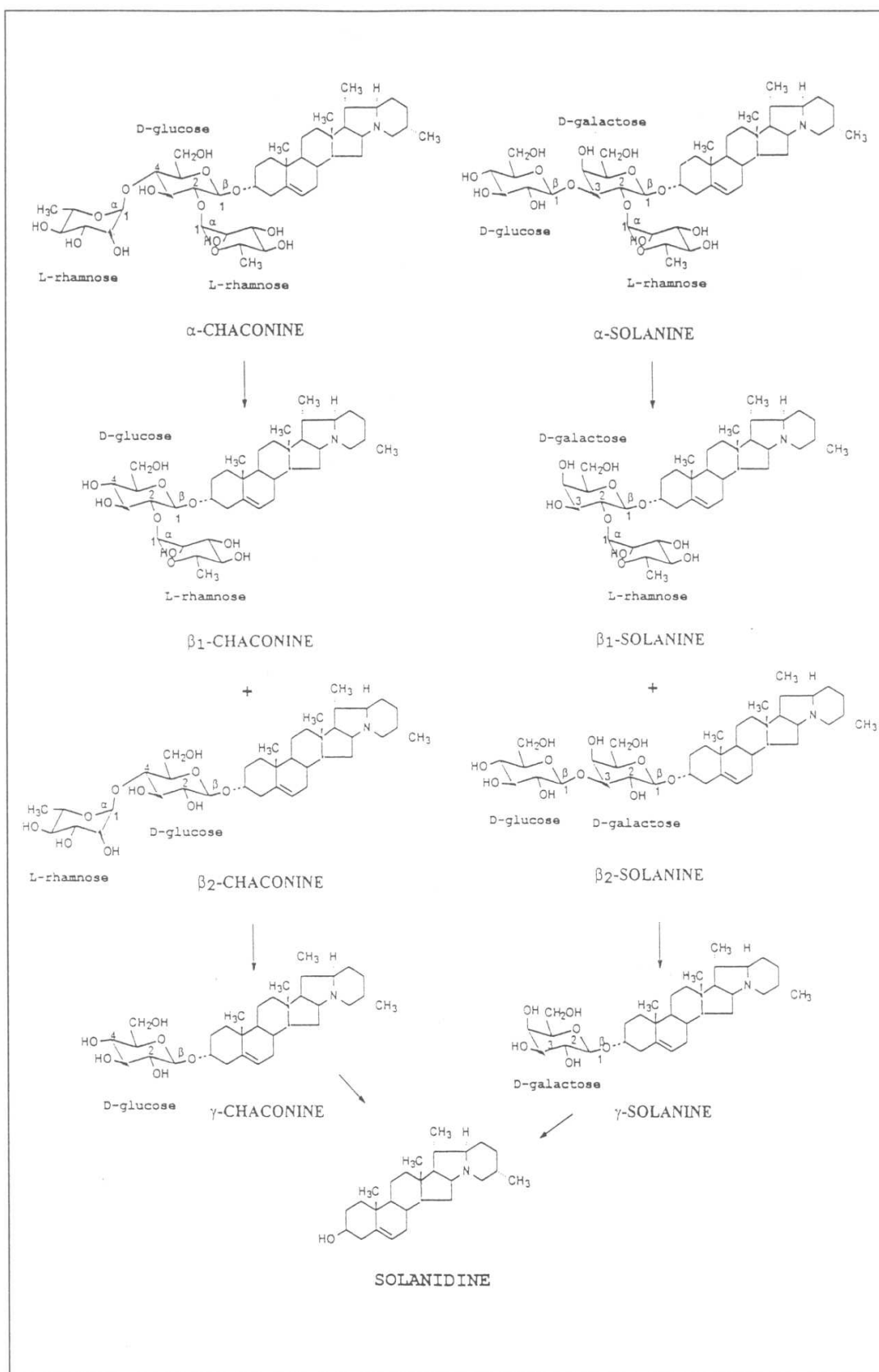


Figure 1.2 Structures of hydrolysis products of  $\alpha$ -chaconine and  $\alpha$ -solanine.



three phases of elimination were identified in plasma with half-lives of 2 to 3.7 min, 2 to 5 h, and 72 to 104 h, respectively. Low rates of tritium excretion were seen in urine and faeces; together they accounted for about 5% of the administered dose during the first 24 h. After this time, the rate of tritium elimination from the body was only about 1-2% a day, corresponding to an overall half-life of 34 to 68 days. The radiochemical data alone do not rigorously prove that solanidine (or its possible metabolite), is retained within the human body, but only that tritium is retained. It is conceivable that a tritium-containing fragment might be cleaved from the solanidine molecule and incorporated into another molecule having a very low turnover. It is not possible to exclude this mechanism but the presence of solanidine in post-mortem livers underscores that a part of the retained tritium fraction might be represented by solanidine [39]. A food study in the UK, in which volunteers were exposed to GAs via potato products, assumed a terminal half-life of solanidine of approximately 72-96 hours [40].

#### **1.4.4 Multiple dose kinetics versus single dose kinetics**

In a study in the UK in humans, solanidine concentrations have been measured. In this study blood samples were collected from healthy males and females in the UK (during the winter) eating their usual diets [40]. Serum samples were collected before the midday meal (time after consumption of last potato product is not given) and were analysed for solanidine. In males the mean level of solanidine was 10.8 ng/ml (27 nmol/l with a range of 2.1- 22.5 ng/ml (5.3-56.3 nmol/l), whereas in females the respective values were 7.9 ng/ml (19.7 nmol/l) with a range of 1.6 -18.5 ng/ml (4.0-46.3 nmol/l). For both genders there was a significant correlation between serum solanidine levels and the estimated alkaloid intake.

In a second study, blood samples were collected from healthy males and females in the UK (during the summer) and in Sweden eating their usual diets or a diet with a high GA-content (200-300 g, cooked, unpeeled potato: mean 240 mg GA/kg) daily for 1 week before sampling [41]. Serum concentrations of English and Swedish subjects eating their usual diet ranged from 3.2 to > 125 nmol/l for total alkaloid and 2.5 to 92.5 nmol/l for solanidine and were comparable in both populations. Serum alkaloid concentrations were significantly raised in the Swedish subjects eating the diet with a high GA-content (125 nmol/l (mean, n=3) for total alkaloid and 80.8 nmol/l (mean, n=3) for solanidine).

### **1.5 Study objectives**

The limit of 200 mg GA/kg potatoes is based on a small amount of evidence since proper information on GA toxicity or pharmacokinetics in humans is lacking. There have been some studies in various species of animals, but due to interspecies differences, these data are not extrapolatable to the human situation. Laboratory animals appear to be less sensitive to GA toxicity than man. In order to find out whether these differences are due to differences in pharmacokinetics or in pharmacodynamics, first pharmacokinetics of  $\alpha$ -solanine,  $\alpha$ -chaconine and solanidine need to be further investigated in humans. The limited information on GA toxicity in relation to dose in humans urges the need to first perform a dose finding study (pilot

study) to find an optimal dose and sampling scheme for a subsequent study on the pharmacokinetics of orally administered GAs in humans. With the gathered information of the full study, toxicity data from animal studies can be interpreted and will enable health policy makers to make a conscious decision, whether or not the present recommended limit– of 200 mg/kg potatoes – needs adaptation.

## **1.6 Outline of report**

This report describes the results of a pilot study on the pharmacokinetics of orally administered  $\alpha$ -solanine and  $\alpha$ -chaconine in healthy volunteers. Chapter 2 of this report primarily describes the study design of the pilot study, the subjects, GA treatments, and analytical procedure for determining  $\alpha$ -solanine and  $\alpha$ -chaconine in human serum. Effects of dosage form and dose height on the pharmacokinetics, and the occurrence of adverse/toxic effects are presented in the results section in chapter 3. In chapter 4, the results are discussed and the most optimal GA dose and blood sampling scheme for the full study (to be performed in future) is given. Furthermore, some suggestions for the full study are given in chapter 4. Conclusions are presented in chapter 5.

## 2. Materials & Methods

### 2.1 Rationale for the design of the pilot study

To attain the objective of the pilot study (to obtain an optimal GA dose and blood sampling scheme for the full study) two criteria are important:

- 1) The optimal sampling scheme and optimal GA dose should result in serum concentration time curves of GAs, that enable adequate description of the basic pharmacokinetics of GAs;
- 2) The optimal GA dose should not result in gastrointestinal effects or other toxic effects.

The general way to study basic pharmacokinetics (including  $C_{\max}$ ,  $t_{\max}$ , internal exposure ( $AUC_{0-\infty}$  of serum concentration time curve), terminal halflife and oral bioavailability) of a compound is after oral and intravenous administration of a solution containing the compound of interest. Unfortunately, it was not possible to dose the GAs intravenously, since too limited information on toxicological margins of these compounds was available. Moreover, overdosing may easily lead to very serious effects. Alternatively, it was decided to study basic pharmacokinetics of GAs after oral administration of a solution containing  $\alpha$ -solanine and  $\alpha$ -chaconine and after oral administration of potatoes containing a defined amount of  $\alpha$ -solanine and  $\alpha$ -chaconine.

In view of the limited information on toxicity of GAs and its corresponding dose in man it was decided to perform a dose escalation study and to start with a conservative GA dose for both treatments. This resulted in a study design in which 1) half of the study population received a solution containing increasing concentrations of  $\alpha$ -solanine and  $\alpha$ -chaconine and the other half received an increasing portion of mashed potatoes containing known amounts of  $\alpha$ -solanine and  $\alpha$ -chaconine and 2) adverse/toxic effects were strictly monitored after each dose administration.

It is also important to investigate the pharmacokinetics of solanidine, since the site of metabolism is not clear and the contribution of solanidine to toxic effects of GAs is unknown. Unfortunately, it was not possible to determine solanidine in this pilot study due to analytical problems. The analytical method will however be optimised furtherly allowing determination of this compound in the full study.

### 2.2 Test materials

The test materials used in this study were 1) a test solution containing  $\alpha$ -solanine and  $\alpha$ -chaconine and 2) mashed potatoes.

For each test period, the test solution was freshly prepared by the Central Pharmacy of the Utrecht University Hospital. The test solutions were prepared by dissolving the proper amounts of  $\alpha$ -solanine (CAS 20562-02-1, purity >99%) and  $\alpha$ -chaconine (CAS 20562-03-2, purity >99%), supplied by Fluka, in slightly acid distilled water. The GA concentration of the test solution for each test period increased with the GA-dose to be administered (see 3.2). The GA composition was 50%  $\alpha$ -solanine and 50%  $\alpha$ -chaconine. The  $\alpha$ -solanine and  $\alpha$ -chaconine

concentrations of the test solutions have been checked by means of the same analytical procedure as applied for measurement of serum samples.

Potato flakes were prepared in co-operation with the Agrotechnical Research Institute (ATO-DLO) Wageningen. A potato variety (Elkana) containing relatively high amounts of GAs was steam peeled, boiled, mashed and dried to obtain potato flakes. The potato flakes were canned under nitrogen atmosphere and stored at 4°C. The production process has been inspected by a RIVM (NVIC) quality assurance co-ordinator.

For each test period, mashed potatoes containing  $\alpha$ -solanine and  $\alpha$ -chaconine were freshly prepared from potato flakes conform strict recipe. Tinned potato flakes were mixed with hot tap water (90°C) an hour before consumption by the volunteers. Appendix 1 lists the composition and preparation of the administered mashed potatoes. The mashed potatoes contained 199 mg GA/kg: 101 mg  $\alpha$ -solanine/kg (51% of total GA) and 98 mg  $\alpha$ -chaconine/kg (49% of total GA). During each test period the  $\alpha$ -chaconine and  $\alpha$ -solanine contents of a sample of mashed potatoes have been checked by means of a method applying high performance liquid chromatography (HPLC) using UV detection by the Laboratory for Residue Analysis (ARO) (ARO SOP 428).

## 2.3 Subjects

A sample size of minimal 2 and maximal 3 participants was planned to be used for each dose step and treatment. Volunteers had to meet the following criteria:

- Age 18 – 45 years, male or female
- Provision of written informed consent
- Good health according to the clinical investigator
- Willingness and ability to adhere to the regimen of the study
- Body weight between 60 and 80 kg (this criterium has not been met for every volunteer: two volunteers with a body weight of 82 kg have been included in the study).
- Quetelet-index (weight {in kg}/square height {in m}) between 20 and 27.

The population tested consisted of 9 men and 10 women, age 20-28 and 20-34, respectively. Body weight ranged from 66 to 82 kg in men and 60 to 79 kg in women.

## 2.4 General design

The design characteristics of this pilot study can be summarised as follows: open, dose escalation study using two different formulations. For each formulation four different dose steps were planned (GA dose steps for mashed potatoes: 0.80, 0.95, 1.10, and 1.25 mg/kg body weight; GA dose steps for test solution: 0.20, 0.30, 0.40 and 0.50 mg/kg body weight ). Each dose was administered after overnight fasting. Each participant received only one single dose of either mashed potatoes or test solution. The maximal consumption period was 30 minutes for both mashed potatoes and test solution.

The starting dose of the first administration of mashed potatoes was based on the present allowable GA-concentration in potatoes in the Netherlands (200 mg GA per kg potatoes), calculated for an adult with 57.5 kg body weight and an average consumption of 230 g potatoes. Body weight dosing implicates that the amount of mashed potatoes to be consumed in this pilot study increased with increasing body weight.

Since an oral solution was expected to induce adverse effects at a lower dose, a conservative dose of 0.20 mg/kg body weight was chosen as starting dose.

Pharmacokinetics of  $\alpha$ -solanine and  $\alpha$ -chaconine were evaluated after each dose step.

To monitor possible adverse/toxic effects, the following parameters have been included in the study: observation of volunteers during the first 24 hours of the study, measurement of vital signs (measurement of blood pressure and heart rate, electro-cardiography), and measurement of blood cholinesterase (to measure possible inhibition of acetyl-cholinesterase by GAs because GAs are known inhibitors of acetyl-cholinesterase).

## 2.5 Criteria for stopping the study

Since this pilot study was a dose escalation study, a set of stopping criteria was formulated. Higher doses would not be administered in the pilot study if:

- The observed concentration-time curves enabled an adequate description of the pharmacokinetic profile (concentrations well above the detection limit) and no adverse effects occurred.
- Light gastrointestinal effects occurred in the form of vomiting and/or diarrhoea. The reason to stop at the light gastrointestinal effect stage are twofold. First, to exclude the possibility of systemic effects at higher doses. Second, because these light gastrointestinal effects directly influence the pharmacokinetic results of the pilot study.

## 2.6 Dietary rules

On every day of examination, participants were asked to complete a short diet questionnaire concerning GA containing consumption products. With the exception of the administered test products, consumption products containing potatoes or potato flour were prohibited in the 72 hour period preceding each product administration and during the entire study.

On the evening before each treatment, participants were instructed to refrain from eating or drinking (with the exception of water) after 23.00 hours.

In view of the results of the studies described in paragraph 1.4, a 72 hours period of potato abstinence preceding each product administration was chosen, in order to warrant that  $\alpha$ -solanine and  $\alpha$ -chaconine would be almost completely eliminated before product administration. This period was based on terminal elimination half lives of  $\alpha$ -solanine and  $\alpha$ -chaconine as described by Hellenäs et al. [37].

## 2.7 Study procedures

For each participant, the study was performed over a period of 5 days. Each of the 19 participants stayed at the Clinical Research Unit for the first 24-hour period post-dosing. Thereafter, they attended the Unit five times at 32, 48, 56, 72, and 96 hours postdose.

Table 2.1 presents a schedule of study procedures for the pilot dose-finding study (with a maximum of 4 dose steps). The study procedures were the same for all test material administrations.

*Table 2.1 Schedule of study procedures.*

Procedure	Check-in	Day 0	Day 1 - 4	Check-out
Product administration		X		
Vital signs and adverse events	X	X	X	X
Blood sampling for GA pharmacokinetics		X	X	
Blood sampling for biochemical parameters*				
- cholinesterase	X	X	X	X
- other parameters	X			X
Blood sampling for hematological tests*	X			X
Medical history	X	X	X	X
Physical examination **	X	X*	X*	X
Pregnancy test		X		
Urine testing*	X			X

\*see appendix 5

\*\*If indicated by medical history

### 2.7.1 Product administration

At day 0, diet adherence was registered and consequently mashed potatoes or test solution was administered at approximately 09.00 a.m. In order to optimize gastric passage, participants were instructed to sit up straight in bed for the first four hours after administration.

### **2.7.2 Vital signs and adverse events**

Blood pressure and heart rate were monitored with a non-invasive automated blood pressure meter (Passport Monitor of Datascope®). Twelve-lead electrocardiograms were obtained using a Hewlett Packard cardiograph (type 4700-A).

Participants were regularly asked whether they had any complaints or symptoms to report. Any adverse events observed or reported were documented.

### **2.7.3 Blood sampling for GA pharmacokinetics**

From 1 hour predosing until 96 hours post dosing, frequent blood samplings were performed for determination of pharmacokinetics of GAs. For reasons of convenience (for both participants and clinical investigators) an intravenous cannula was inserted in the participant's forearm. In this way samplings of blood were performed at  $t = -1, -0.5, 0, 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 20$ , and 24 hours, regarding the time point of test product administration as time point zero. Extra blood samples were taken at 0.5 and 1.5 hours following administration of the test solution. After the first 24-hour post dose time period, the intravenous cannula was removed. Further blood samplings were performed at 32, 48, 56, 72, and 96 hours post dose by venepuncture during the return visits. Serum samples were obtained after complete precipitation of the cellular fraction (at room temperature). Serum samples were stored at  $-20^{\circ}\text{C}$  until analysis for GAs.

### **2.7.4 Blood sampling for cholinesterase**

Blood was sampled during the check-in, and check-out and at  $t = -1, 7$  and 24 hours for measurement of blood cholinesterase levels.

### **2.7.5 Other study procedures**

For detailed information on the other study procedures mentioned in table 2.1 is referred to the clinical report 'Clinical pilot study on the bioavailability of glycoalkaloids from potato' Earl Johanns and Tjeert Mensinga, March 2001 (this report is included in appendix 5).

## **2.8 Analysis of blood samples**

### **2.8.1 Analysis of GAs in human serum**

At the Laboratory of Exposure Assessment & Environmental Epidemiology a high performance liquid chromatography (HPLC) method was developed for the separation and quantification of  $\alpha$ -solanine and  $\alpha$ -chaconine in human blood serum. A method previously described by Hellenäs et al. was adjusted for application in pharmacokinetic studies [37]. This adjustment contained mainly automation of the analysis. In appendix 2 a schematic overview of the analysis is given. For detecting the GAs, serum was first cleaned up by a manual solid-phase extraction followed by a second clean up on an analytical cyanopropyl column. Subsequently, the compounds were separated and measured on an analytical system

equipped with a silica-column. Because of the low sensitivity of the GAs and the low concentration of the GAs in the blood samples, a sample enrichment column instead of the loop of the injector was used in both the cyanopropyl-system for clean up as the analytical silica-system (see appendix 2).

The analytical procedure was validated. The lower limit of quantification (LLQ) for  $\alpha$ -chaconine was 0.25 ng/ml serum and for  $\alpha$ -solanine 0.50 ng/ml serum. The method was linear up to 50 ng/ml serum for both compounds. Precision was determined at 3 concentration levels (12.5 - 20 en 40 ng/ml) at 4 different days. Within day-variation was less than 10% both for  $\alpha$ -chaconine and for  $\alpha$ -solanine. Between day-variation was also less than 10% for both compounds. For stability test, serum obtained from a blood bank was spiked with  $\alpha$ -solanine and  $\alpha$ -chaconine at concentration levels of 2 ng/ml and 25 ng/ml. The spiked samples were stored at  $-20^{\circ}\text{C}$  and measured over a period of 12 weeks. No significant decrease of concentrations could be detected.

### 2.8.2 Analysis of blood cholinesterase

Measurements of blood cholinesterase (see appendix 5) were performed by the Laboratory for Project Research - Utrecht Diagnostics (University Hospital Utrecht), under intralaboratory quality control conditions (using Biorad standard samples) and interlaboratory surveillance conditions (using the so called 'ring validation' method, consisting of regular sample comparisons between a series of laboratories for clinical trials (chemistry) in the Netherlands [under supervision of the 'Stichting Kwaliteitsbewaking Klinisch chemische Ziekenhuislaboratoria']).

## 2.9 Pharmacokinetic parameters

Laboratory analysis of the serum samples provided serum concentration-time profiles for  $\alpha$ -solanine and  $\alpha$ -chaconine for each subject and each treatment. These serum concentration-time data were analysed to determine, model independently, the following kinetic parameters (for each subject, each treatment, for  $\alpha$ -solanine and  $\alpha$ -chaconine):

- $C_{\max}$  - Datapoint with the highest concentration
- $t_{\max}$  - Timepoint corresponding to  $C_{\max}$
- $t_{1/2}$  - Elimination half-life
- $t_{\text{last}}$  - Latest timepoint corresponding to a measurable concentration (above the LLQ)
- $AUC_{0-t_{\text{last}}}$  - Area under the curve (from point zero to  $t_{\text{last}}$  after product administration)
- $AUC_{0-\infty}$  - Area under the curve (from point zero to infinity), approximated by adding  $AUC_{0-t_{\text{last}}}$  and  $AUC_{t_{\text{last}}-\infty}$ . The last parameter is calculated by extrapolation of the area under the curve from  $t_{\text{last}}$  after product administration to infinity by using the rate constant determined in the terminal elimination phase.

The relative bioavailability of  $\alpha$ -solanine and  $\alpha$ -chaconine for treatment A (mashed potatoes) can be estimated by dividing the  $AUC_{0-\infty}$  of treatment A by the  $AUC_{0-\infty}$  of treatment B (test solution), with correction for the administered dose; as presented in the following equation:



$$\frac{(\text{AUC}_{0-\infty} \text{ of treatment A})}{(\text{AUC}_{0-\infty} \text{ of treatment B})} \text{ times } \frac{(\text{Dose of treatment B})}{(\text{Dose of treatment A})}$$

### 3. Results

#### 3.1 Performance of dose steps

The dose steps of the test solution have been changed during the study. After two test periods it appeared from intermediate results that the original dose step scheme for the test solution was too conservative and would not lead to an adequate pharmacokinetic profile. Serum concentrations of the first two periods were lower than expected and could not be followed long enough in time to obtain a serum concentration-time profile suitable for adequate pharmacokinetic analyses. Furthermore, in the first two test periods with mashed potatoes two times higher serum concentrations were measured without adverse effects. In view of these results it was decided to adjust the dose steps for the test solution as follows: GA-dose step 0.40 mg/kg body weight was omitted and GA-dose step 0.70 mg/kg body weight was added. An amendment to the protocol was written and approved by the medical ethical board (of the Utrecht Medical Centre) to implement these dosing changes.

All dose steps of the other treatment, mashed potatoes, were performed. An overview of the final dose steps per treatment is given in figure 3.1.

After each test period the results of the pharmacokinetic analysis and adverse effects were evaluated to check whether the STOP-criteria (light gastrointestinal effects and/or concentration-time curves enabling an adequate description of the pharmacokinetic profile) were met. These criteria have not been met during the study (see figure 3.1).

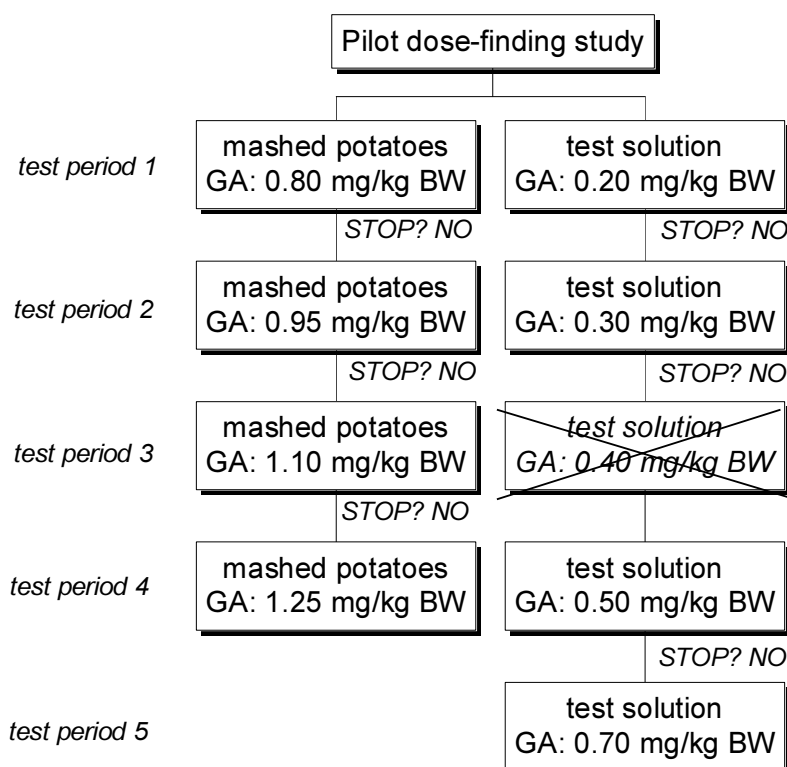


Figure 3.1. Performed dose steps of the pilot study.

## 3.2 Individual GA doses

### 3.2.1 Mashed potatoes

The amounts of mashed potatoes administered to the volunteers ranged from 289 to 453 g (see table 3.1). The GA doses per subject ranged from 58 to 90 mg.

*Table 3.1 Body weight, amounts of mashed potatoes consumed and GA dose for individual subjects treated with mashed potatoes.*

GA dose* (mg/kg body weight)	Subject	Body weight (kg)	Mashed potatoes** (g)	GA dose*/subject (mg)
0.80	12	72	289.0	57.60
	17	79	317.6	63.20
	21	76	305.9	60.80
0.95	03	67	319.8	63.65
	10	70	334.2	66.50
	11	79	377.1	75.05
1.10	15	72	398.0	79.20
	20	71	392.5	78.10
	22	82	453.3	90.20
1.25	09	70	439.7	87.50
	14	72	452.3	90.00

\* GA dose consisted of 51%  $\alpha$ -solanine and 49%  $\alpha$ -chaconine.

\*\*Mashed potatoes contained 199 mg GA/kg: 101 mg  $\alpha$ -solanine/kg and 98 mg  $\alpha$ -chaconine/kg.

### 3.2.2 Test solution

The volume of test solution administered to the volunteers ranged from 188 ml to 256 ml. The GA doses per subject ranged from 13 to 50 mg (see table 3.2).

*Table 3.2 Body weight, volumes of test solution consumed and GA dose for individual subjects treated with test solution.*

GA dose* (mg/kg body weight)	Subject	Body weight (kg)	Test solution**		GA dose*/subject (mg)
			mg GA*/ml	ml	
0.20	04	66	0.064	206.3	13.20
	06	82		256.3	16.40
0.30	18	66	0.096	206.3	19.80
	19	61		190.6	18.30
0.50	24	65	0.160	203.1	32.50
	25	60		187.5	30.00
0.70	05	71	0.224	221.9	49.70
	23	62		193.8	43.40

\* GA dose consisted of 50%  $\alpha$ -solanine and 50%  $\alpha$ -chaconine.

\*\*Each test solution contained 50%  $\alpha$ -solanine and 50%  $\alpha$ -chaconine.

### 3.3 GA serum concentration-time profiles

The quality of the serum samples of the first test period (mashed potatoes: 0.80 mg/kg body weight ; GA-solution 0.2 mg/kg body weight) was unsatisfactory due to the use of plastic tubes for blood collection (plastic tubes were chosen to prevent adsorption of GA to the tube). To obtain good serum samples, the cellular fraction of the blood samples should precipitate completely. This precipitation process was not complete in the plastic tubes. It was decided to use glass tubes instead for the following test periods.

Most of the GA serum concentrations of the first test period could not be measured. Therefore, GA serum concentration-time profiles could not be used for adequate pharmacokinetic analyses. The GA serum concentration-time profiles of this period are not included in this report. The first test period nevertheless showed maximum concentrations of  $\alpha$ -solanine and  $\alpha$ -chaconine between 4 and 12 hours. Therefore, it was decided to adapt the blood sampling scheme for both treatments: time point t=10 hours was added to obtain more precise results of the  $\alpha$ -solanine and  $\alpha$ -chaconine peak in the serum concentration-time profile.

$\alpha$ -Solanine serum concentration-time profiles for each subject and for each treatment (test period 2-5) are shown in figure 3.2;  $\alpha$ -chaconine serum concentration-time profiles for each subject and for each treatment (test period 2-5) are shown in figure 3.3. During each test period serum samples up to 96 hours after administration were analysed. As can be seen from figure 3.2 it was not possible to measure serum concentrations of  $\alpha$ -solanine up to 96 hours: serum  $\alpha$ -solanine concentrations could be measured up to 48-56 hours for mashed potatoes, and up to 24-32 hours for test solution. For  $\alpha$ -chaconine, serum concentration profiles could be followed longer in time (mashed potatoes: up to 72-96 h; test solution: up to 56-96 hours). The 96-hours time point could be quantified in five out of fourteen volunteers (mashed potatoes (GA dose 1.10 mg/kg body weight): two volunteers, (GA dose 1.25 mg/kg body weight) two volunteers; test solution (GA dose 0.50 mg/kg body weight): one volunteer).

In the HPLC-chromatograms of the serum samples of testperiod 3 (mashed potatoes, GA: 1.10 mg/kg body weight) and 4 (mashed potatoes, GA: 1.25 mg/kg body weight and test solution GA: 0.50 mg/kg body weight) an extra peak was observed, which seemed to be related to  $\alpha$ -solanine or  $\alpha$ -chaconine. Unfortunately, this peak could not be identified and was therefore entitled 'ghost peak'(see appendix 3). The ghost peak has been quantified either as  $\alpha$ -solanine or as  $\alpha$ -chaconine. The possible effect of the ghost peak on the serum concentration time profile of  $\alpha$ -solanine and  $\alpha$ -chaconine in a volunteer, who received 1.25 mg/kg mashed potatoes, is shown in figure 3.4. In this volunteer, the ghost peak had the most pronounced effect on the serum concentration time profile of  $\alpha$ -solanine and  $\alpha$ -chaconine.

In addition, subject 14 vomited at 3.45 hours post dosing (see 3.5). This might have influenced the serum GA levels because gastric emptying of mashed potatoes might not have been complete at that time and part of the GA dose might have been lost by vomiting, resulting in lower serum GA levels.

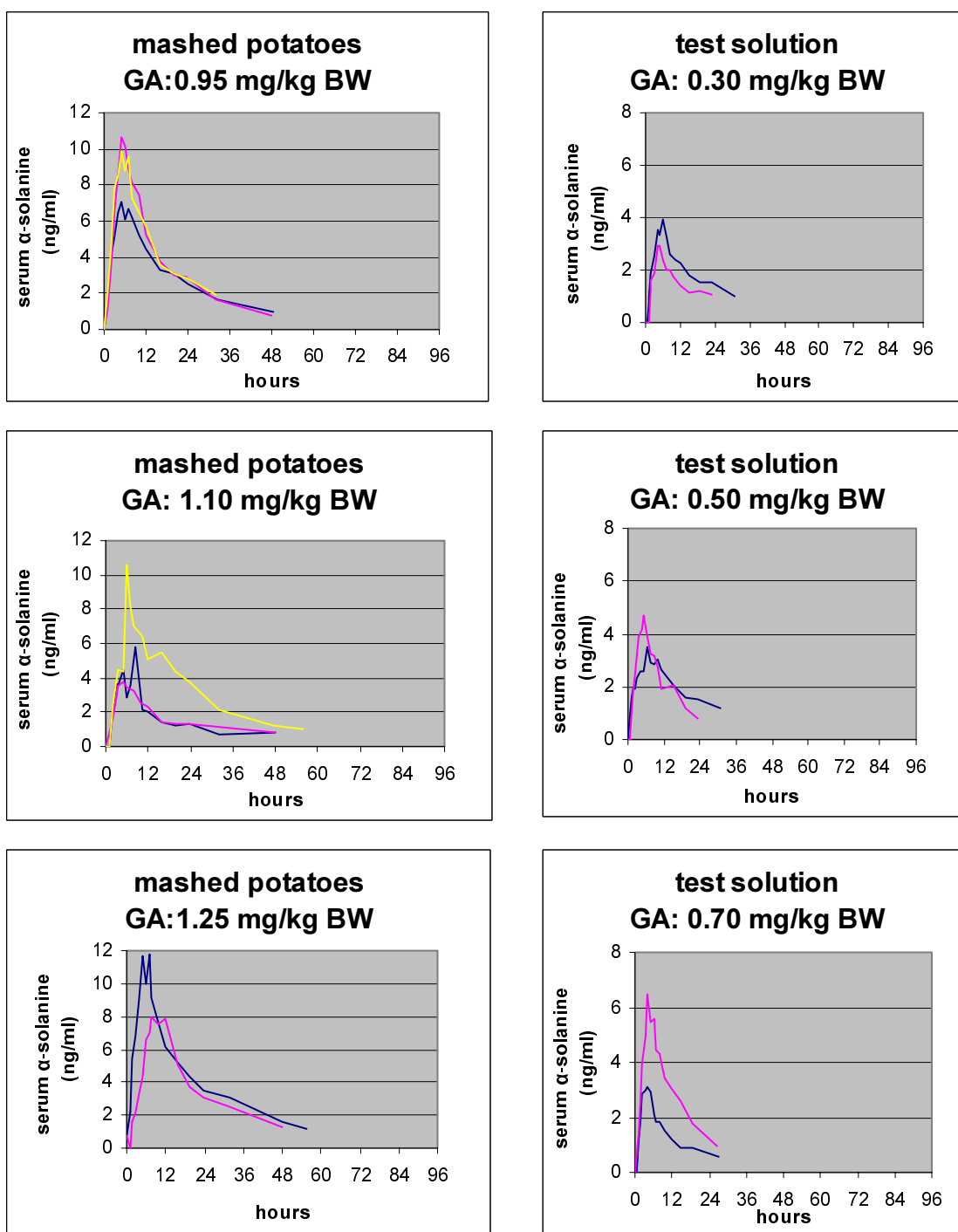


Figure 3.2  $\alpha$ -Solanine serum concentrations as function of time in healthy human volunteers ( $n=2-3$ ) after ingestion of mashed potatoes (GA doses: 0.95, 1.10, and 1.25 mg/kg body weight) or test solution (GA doses: 0.30, 0.50, and 0.70 mg/kg body weight). For each subject serum samples have been analysed up to 96 hours after administration. Serum  $\alpha$ -solanine concentrations below LLQ (0.50 ng/ml) are not included. (BW= body weight).

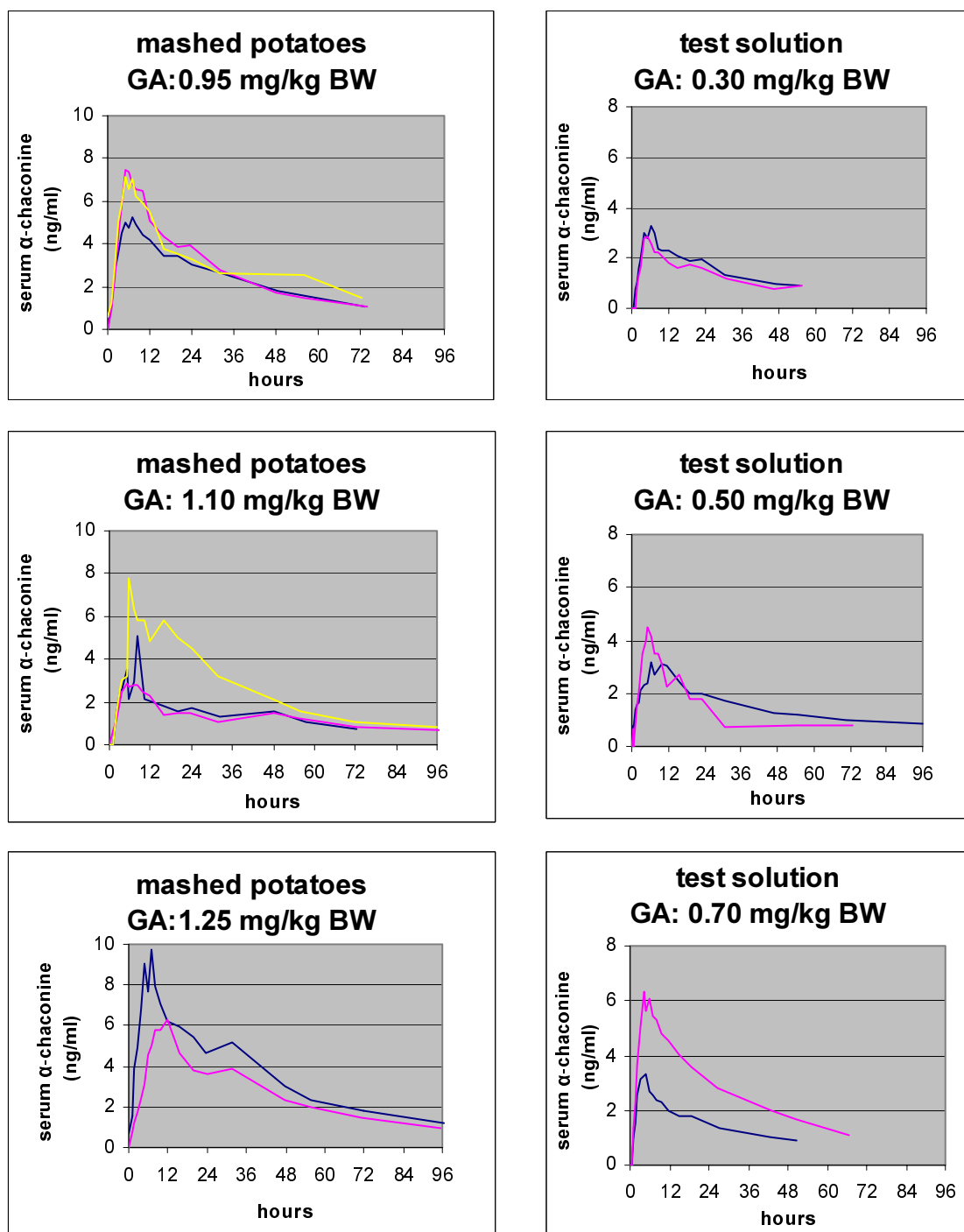


Figure 3.3  $\alpha$ -Chaconine serum concentrations as function of time in healthy human volunteers ( $n=2-3$ ) after ingestion of mashed potatoes (GA doses: 0.95, 1.10, and 1.25 mg/kg body weight) or test solution (GA doses: 0.30, 0.50, and 0.70 mg/kg body weight). For each subject serum samples have been analysed up to 96 hours after administration. Serum  $\alpha$ -chaconine concentrations below LLQ (0.25 ng/ml) are not included. (BW= body weight).

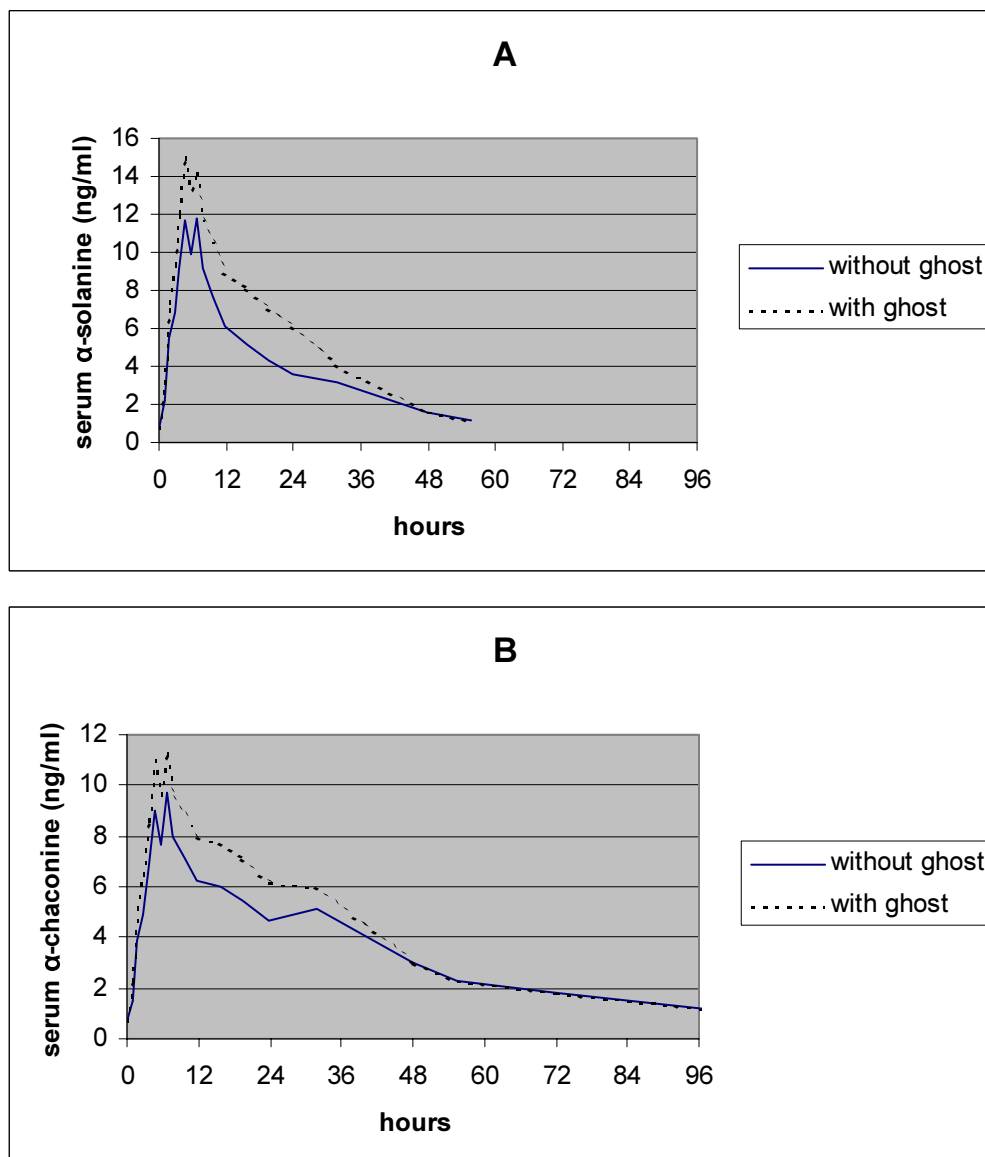


Figure 3.4 Effect of the ghost peak on  $\alpha$ -solanine and  $\alpha$ -chaconine serum concentration-time profiles of a healthy human volunteer who ingested mashed potatoes (GA-dose: 1.25 mg/kg body weight). **A)** effect of quantification of the ghost peak as  $\alpha$ -solanine; **B)** effect of quantification of the ghost peak as  $\alpha$ -chaconine.

### 3.4 Pharmacokinetic analysis

$\alpha$ -Solanine and  $\alpha$ -chaconine serum concentration-time profiles of testperiod 2-5 were analysed to determine the basic pharmacokinetic parameters  $C_{\max}$ ,  $t_{\max}$ ,  $t_{1/2}$ ,  $AUC_{0-\infty}$  (the parameters  $AUC_{0-t_{\text{last}}}$  and  $t_{\text{last}}$  have been determined to calculate  $AUC_{0-\infty}$ ). In addition, the relative bioavailability of mashed potatoes versus test solution ( $F_{\text{rel}}$ ) has been calculated in two ways: using  $AUC_{0-\infty}$  (see 2.9), and using  $AUC_{0-24\text{h}}$ .  $AUC_{0-24\text{h}}$  was chosen since  $\alpha$ -solanine could be measured for all groups at  $t=24$  hours.

Although the pharmacokinetic parameters have been determined for test period 2-5 this does not implicate that the observed serum concentration-time curves enabled an adequate determination of all of the pharmacokinetic parameters for each of these test periods (see below).

In the following, an overview of the pharmacokinetic parameters that could be determined adequately is presented in table 3.3. In addition, the results of each pharmacokinetic parameter are discussed. The individual basic pharmacokinetic data of  $\alpha$ -solanine and of  $\alpha$ -chaconine are shown in table 3.4 and 3.5, respectively. The data of  $F_{\text{rel}}$  of  $\alpha$ -solanine are presented in table 3.6 A, those of  $\alpha$ -chaconine in table 3.6 B.

*Table 3.3 Achievement of required pharmacokinetic parameters in the pilot study.*

Test formulation	GA dose (mg/kg body weight)	Required pharmacokinetic parameters	Satisfactory for $\alpha$ -solanine?	Satisfactory for $\alpha$ -chaconine?
mashed potatoes	0.95	$C_{\max}$ $t_{\max}$ $t_{1/2}$ $AUC_{0-\infty}$	Yes Yes No (< LLQ) No (< LLQ)	Yes Yes Yes Yes
	1.10	$C_{\max}$ $t_{\max}$ $t_{1/2}$ $AUC_{0-\infty}$	Yes Yes No (ghost peak, < LLQ) No (< LLQ)	Yes Yes No (ghost peak) Yes
	1.25	$C_{\max}$ $t_{\max}$ $t_{1/2}$ $AUC_{0-\infty}$	No (ghost peak, vomiting) No (ghost peak, vomiting) No (< LLQ) No (ghost peak, vomiting, < LLQ)	No (ghost peak, vomiting) No (vomiting) Yes No (ghost peak, vomiting)
test solution	0.30	$C_{\max}$ $t_{\max}$ $t_{1/2}$ $AUC_{0-\infty}$	Yes Yes No (< LLQ) No (< LLQ)	Yes Yes No (< LLQ) No (< LLQ)
	0.50	$C_{\max}$ $t_{\max}$ $t_{1/2}$ $AUC_{0-\infty}$	Yes Yes No (< LLQ) No (< LLQ)	Yes Yes Yes Yes
	0.70	$C_{\max}$ $t_{\max}$ $t_{1/2}$ $AUC_{0-\infty}$	Yes Yes No (< LLQ) No (< LLQ)	Yes Yes No (< LLQ) No (< LLQ)



### 3.4.1 $C_{\max}$ and $t_{\max}$

For both  $\alpha$ -solanine and of  $\alpha$ -chaconine, blood sampling during the first hours after administration of mashed potatoes and test solution was frequent enough, and the GA doses were high enough, to determine the peak and its top (described by  $C_{\max}$  and  $t_{\max}$ ) adequately from the serum concentration-time profiles of each subject. Data of subject 14 were found to be questionable because this subject vomited before the top of the peak had been reached. In addition,  $C_{\max}$  of test periods in which a ghost peak was observed (see 3.3) might be higher (3.1 ng/ml at most for  $\alpha$ -solanine and 1.5 ng/ml at most for  $\alpha$ -chaconine (subject 14 excluded)) (see table 3.4 and 3.5, respectively).

After administration of mashed potatoes (GA dose: 0.95, 1.10, and 1.25 mg/kg body weight),  $C_{\max}$  of  $\alpha$ -solanine ranged between 3.8 and 11.8 ng/ml (ghost peak and subject 14 excluded) and  $C_{\max}$  of  $\alpha$ -chaconine ranged between 2.9 and 9.7 ng/ml (ghost peak and subject 14 excluded). Furthermore the data demonstrate that for each subject,  $C_{\max}$  of  $\alpha$ -solanine was always higher than  $C_{\max}$  of  $\alpha$ -chaconine. The highest concentration of  $C_{\max}$  for both  $\alpha$ -solanine and  $\alpha$ -chaconine is observed in subject 9 of the highest dose group.

After administration of test solution (GA dose: 0.30, 0.50 and 0.70 mg/kg body weight),  $C_{\max}$  of  $\alpha$ -solanine and  $\alpha$ -chaconine were lower than after administration of mashed potatoes.  $C_{\max}$  of  $\alpha$ -solanine ranged between 2.9 and 6.5 ng/ml (ghost peak excluded) and  $C_{\max}$  of  $\alpha$ -chaconine ranged between 2.8 and 6.3 ng/ml (ghost peak excluded). In contrast to the group that consumed mashed potatoes,  $C_{\max}$  of  $\alpha$ -solanine and  $\alpha$ -chaconine were roughly the same for each individual. The highest concentration of  $C_{\max}$  for both  $\alpha$ -solanine and  $\alpha$ -chaconine is observed again in one of the two subjects of the highest dose group.

After administration of mashed potatoes (GA dose: 0.95, 1.10 and 1.25 mg/kg body weight),  $t_{\max}$  of  $\alpha$ -solanine and  $\alpha$ -chaconine were within the same range (5-8 hours) (ghost peak and subject 14 excluded). After administration of test solution (GA dose: 0.30, 0.50 and 0.70 mg/kg body weight),  $t_{\max}$  of  $\alpha$ -solanine ranged between 4 and 6 hours (ghost peak excluded). A similar range was obtained for  $t_{\max}$  of  $\alpha$ -chaconine (ghost peak excluded).  $T_{\max}$  of both glycoalkaloids was relatively long for a solution. In most of the subjects treated with either mashed potatoes or test solution,  $t_{\max}$  of  $\alpha$ -solanine and  $t_{\max}$  of  $\alpha$ -chaconine were reached at roughly the same time points (ghost peak and subject 14 excluded).

The findings of the pilot study regarding  $C_{\max}$  and  $t_{\max}$  of  $\alpha$ -solanine and  $\alpha$ -chaconine after consumption of a mashed potato meal are in agreement with the (sparsely) available pharmacokinetic data of GAs reported by Hellenäs et al [37]. They performed a study in seven healthy male volunteers in which blood serum levels of  $\alpha$ -solanine,  $\alpha$ -chaconine and solanidine were measured during a 25-hours period following a single meal of mashed potatoes (equivalent to 1 mg GAs/kg body weight (1 mg GAs consisted of 0.4 mg  $\alpha$ -solanine and 0.6 mg  $\alpha$ -chaconine)). The mashed potato used in the study of Hellenäs et al. contained almost the same total amount of GAs as the mashed potato used in the pilot study, but the proportion of  $\alpha$ -solanine and  $\alpha$ -chaconine differed between both studies (study of Hellenäs et al.: 200 mg GA/kg mashed potato (82  $\alpha$ -solanine/kg and 118 mg  $\alpha$ -chaconine/kg); pilot study:

Table 3.4A. Pharmacokinetic parameters for  $\alpha$ -solanine: *mashed potatoes*

<b>GA dose: 0.95 mg/kg body weight</b>							
<i>subject</i>	$C_{max}$ (ng/ml serum)	$t_{max}$ (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng*h/ml)	$AUC_{0-t_{last}}$ (ng*h/ml)	$t_{last}$ (h)	$AUC_{0-24h}$ (ng*h/ml)
03	7,0	5	18,1	164	139	48	100
10	10,7	5	13,8	178	163	48	124
11	9,8	5	17,9	193	142	32	124
<b>GA-dose: 1.10 mg/kg body weight</b>							
<i>subject</i>	$C_{max}$ (ng/ml serum)	$t_{max}$ (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng*h/ml)	$AUC_{0-t_{last}}$ (ng*h/ml)	$t_{last}$ (h)	$AUC_{0-24h}$ (ng*h/ml)
15	5,8	8	35,8	111	74	48	53
15+ghost	6,2	5	15,6	142	123	48	86
20	3,8	5	41,7	126	75	48	49
20+ghost	5,8	5	14,4	148	131	48	87
22	10,6	6	16,2	200	178	56	119
22+ghost	10,6	6	22,5	236	203	56	140
<b>GA-dose: 1.25 mg/kg body weight</b>							
<i>subject</i>	$C_{max}$ (ng/ml serum)	$t_{max}$ (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng*h/ml)	$AUC_{0-t_{last}}$ (ng*h/ml)	$t_{last}$ (h)	$AUC_{0-24h}$ (ng*h/ml)
09	11,8	7	19,1	256	223	56	149
09+ghost	14,9	5	19,8	343	309	56	211
14	8,0	8	18,2	204	170	48	118
14+ghost	10,8	12	17,9	305	252	48	169

Table 3.4B. Pharmacokinetic parameters for  $\alpha$ -solanine: *test solution*

<b>GA dose: 0.30 mg/kg body weight</b>							
<i>subject</i>	$C_{max}$ (ng/ml serum)	$t_{max}$ (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng*h/ml)	$AUC_{0-t_{last}}$ (ng*h/ml)	$t_{last}$ (h)	$AUC_{0-24h}$ (ng*h/ml)
18	3,9	6	17,4	84	58	31	48
19	2,9	5	34,5	89	35	23	35
<b>GA-dose: 0.50 mg/kg body weight</b>							
<i>subject</i>	$C_{max}$ (ng/ml serum)	$t_{max}$ (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng*h/ml)	$AUC_{0-t_{last}}$ (ng*h/ml)	$t_{last}$ (h)	$AUC_{0-24h}$ (ng*h/ml)
24	3,5	6	27,9	110	61	31	51
24+ghost	4,6	6	27,9	122	73	31	63
25	4,7	5	5,4	57	51	23	51
25+ghost	5,0	7	5,8	62	55	23	55
<b>GA-dose: 0.70 mg/kg body weight</b>							
<i>subject</i>	$C_{max}$ (ng/ml serum)	$t_{max}$ (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng*h/ml)	$AUC_{0-t_{last}}$ (ng*h/ml)	$t_{last}$ (h)	$AUC_{0-24h}$ (ng*h/ml)
05	3,1	4	17,0	50	35	27	35
23	6,5	4	8,4	86	74	27	74

Table 3.5A. Pharmacokinetic parameters for  $\alpha$ -chaconine: *mashed potatoes*

<b>GA dose: 0.95 mg/kg body weight</b>							
<i>subject</i>	$C_{max}$ (ng/ml serum)	$t_{max}$ (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng*h/ml)	$AUC_{0-t_{last}}$ (ng*h/ml)	$t_{last}$ (h)	$AUC_{0-24h}$ (ng*h/ml)
03	5,2	7	33,6	237	184	74	89
10	7,4	5	27,2	250	212	74	112
11	7,2	5	48,9	345	227	72	109
<b>GA-dose: 1.10 mg/kg body weight</b>							
<i>subject</i>	$C_{max}$ (ng/ml serum)	$t_{max}$ (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng*h/ml)	$AUC_{0-t_{last}}$ (ng*h/ml)	$t_{last}$ (h)	$AUC_{0-24h}$ (ng*h/ml)
15	5,1	8	49,3	168	110	72	50
15+ghost	5,1	8	28,5	170	140	72	70
20	2,9	5	49,5	169	125	101	45
20+ghost	4,1	5	44,1	198	160	101	67
22	7,8	6	59,7	316	244	96	112
22+ghost	7,8	6	59,7	331	260	96	124
<b>GA-dose: 1.25 mg/kg body weight</b>							
<i>subject</i>	$C_{max}$ (ng/ml serum)	$t_{max}$ (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng*h/ml)	$AUC_{0-t_{last}}$ (ng*h/ml)	$t_{last}$ (h)	$AUC_{0-24h}$ (ng*h/ml)
09	9,7	7	44,8	419	340	97	143
09+ghost	11,2	7	44,8	473	394	97	181
14	6,3	12	36,5	299	250	96	98
14+ghost	8,1	12	37,5	359	308	96	131

Table 3.5B. Pharmacokinetic parameters for  $\alpha$ -chaconine: *test solution*

<b>GA dose: 0.30 mg/kg body weight</b>							
<i>subject</i>	$C_{max}$ (ng/ml serum)	$t_{max}$ (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng*h/ml)	$AUC_{0-t_{last}}$ (ng*h/ml)	$t_{last}$ (h)	$AUC_{0-24h}$ (ng*h/ml)
18	3,2	6	31,7	130	88	55	48
19	2,8	5	36,5	117	75	56	41
<b>GA-dose: 0.50 mg/kg body weight</b>							
<i>subject</i>	$C_{max}$ (ng/ml serum)	$t_{max}$ (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng*h/ml)	$AUC_{0-t_{last}}$ (ng*h/ml)	$t_{last}$ (h)	$AUC_{0-24h}$ (ng*h/ml)
24	3,2	6	84,3	242	142	96	54
24+ghost	4,0	6	84,3	251	150	96	63
25	4,5	5	48,5	148	101	73	60
25+ghost	5,0	7	48,5	152	105	73	63
<b>GA-dose: 0.70 mg/kg body weight</b>							
<i>subject</i>	$C_{max}$ (ng/ml serum)	$t_{max}$ (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng*h/ml)	$AUC_{0-t_{last}}$ (ng*h/ml)	$t_{last}$ (h)	$AUC_{0-24h}$ (ng*h/ml)
05	3,3	5	38,9	128	79	51	53
23	6,3	4	28,9	227	181	67	106

199 mg GA/kg mashed potato (101 mg  $\alpha$ -solanine/kg and 98 mg  $\alpha$ -chaconine/kg)). In the study of Hellenäs et al. maximum serum concentrations of  $\alpha$ -solanine and  $\alpha$ -chaconine were achieved 4-6 hours and 4-8 hours post dosing, respectively; whereas  $C_{\max}$  ranged between 3.9 and 11.3 ng/ml for  $\alpha$ -solanine and 6.2 and 21.4 ng/ml for  $\alpha$ -chaconine. Our  $C_{\max}$  and  $t_{\max}$  values of  $\alpha$ -solanine after administration of mashed potatoes with a GA dose of 0.95 mg/kg body weight ( $\alpha$ -solanine: 0.48 mg/kg body weight) were within the range reported by Hellenäs et al. (see table 3.4A); our  $C_{\max}$  and  $t_{\max}$  values of  $\alpha$ -chaconine after administration of mashed potatoes with a GA dose of 1.25 mg/kg body weight ( $\alpha$ -chaconine: 0.61 mg/kg body weight) (subject 9) were also within the range reported by Hellenäs et al. (see table 3.5A) [37].

### 3.4.2 $t_{1/2}$

The optimal way to determine elimination half life,  $t_{1/2}$ , is from blood (or serum or plasma) concentration-time data after intravenous administration of the compound of interest. After intravenous administration it can be assured that the elimination phase is represented by the declining part of the concentration time curve and is not 'disturbed' by absorption and distribution of the compound during that phase (this may happen after oral administration). In the present study, for safety reasons, it was not possible to perform an intravenous administration of GAs. Nevertheless, it was decided to calculate  $t_{1/2}$  from the available serum concentration-time curves, to get some insight on the size of this parameter. For an adequate determination of  $t_{1/2}$ , the declining part (more precisely the last part of the tail) of the serum GA concentration-time curves is needed. As can be seen from figures 3.2 and 3.3 the declining part (including the tail) of the serum  $\alpha$ -chaconine concentration-time curves is in most curves clearly present but the last part of the tail of the serum  $\alpha$ -solanine concentration-time curves is missing (data points up to 72-96 hours). If  $t_{1/2}$  is calculated from the last part (tail) of the curve it is most likely that this part represents the terminal elimination phase, without 'disturbance' by absorption (disturbance by distribution cannot be excluded) resulting in an adequately determined  $t_{1/2}$ . From the serum  $\alpha$ -chaconine concentration-time curves it can be concluded that data points up to approximately 72 hours, are needed to determine  $t_{1/2}$  adequately. For  $\alpha$ -solanine the same data points are needed since the profile follows a similar pattern. Unfortunately, for none of the subjects  $\alpha$ -solanine serum samples could be quantified up to 72 hours due to serum concentrations below the LLQ. Since the tail of all serum solanine concentration-time curves shown in figure 3.2 is (partly) missing, it is likely that all the  $t_{1/2}$  values of  $\alpha$ -solanine (both for mashed potatoes and test solution) probably represent an earlier phase (distribution phase), than the terminal elimination phase. Regarding  $\alpha$ -chaconine, serum samples of all subjects who received mashed potatoes could be quantified up to 72-96 hours, resulting in  $t_{1/2}$  values of the terminal elimination phase. After administration of test solution the terminal elimination phase was observed in the concentration-time profiles of half of the subjects (both subjects of GA dose 0.50 mg/kg body weight and one subject of GA dose 0.70 mg/kg body weight). In these subjects  $t_{1/2}$  of the terminal elimination phase could be determined.

After administration of mashed potatoes (GA dose 0.95, 1.10 and 1.25 mg/kg body weight),  $t_{1/2}$  of  $\alpha$ -solanine for most subjects ranged between 13.8 and 19.1 hours (ghost peak excluded). In two subjects (15 and 20) higher values were calculated (35.8 and 41.7 hours, respectively). In these two subjects the ghost peak may clearly influence  $t_{1/2}$ . If the ghost peak was also quantified as  $\alpha$ -solanine in these two subjects, data in the same range as the other subjects were obtained: 15.6 and 14.4 hours, respectively.  $T_{1/2}$  of  $\alpha$ -chaconine was much higher after administration of mashed potatoes compared to  $t_{1/2}$  of  $\alpha$ -solanine: 27.2 - 59.7 hours (see table 3.5A). In one subject (subject 15) a clear effect of the ghost peak on  $t_{1/2}$  was observed: 28.5 hours (with ghost peak) versus 49.3 (without ghost peak) (see table 3.5A).

After administration of test solution (0.30, 0.50 and 0.70 mg/kg body weight)  $t_{1/2}$  of  $\alpha$ -solanine ranged between 5.4 and 34.5 hours (ghost peak excluded) and  $t_{1/2}$  of  $\alpha$ -chaconine ranged between 28.9 and 84.3 hours (ghost peak excluded). No clear influence of the ghost peak was observed for both  $\alpha$ -solanine and  $\alpha$ -chaconine.

### 3.4.3 $AUC_{0-\infty}$

The area under the curve (from point zero to infinity),  $AUC_{0-\infty}$ , is a measure for internal exposure. This parameter is approximated by adding  $AUC_{0-t_{last}}$  and  $AUC_{t_{last}-\infty}$ . The last parameter is calculated by extrapolation of the area under the curve from  $t_{last}$  after product administration to infinity by using the rate constant determined in the terminal elimination phase.

As shown above, the terminal elimination phase has been reached in none of the serum  $\alpha$ -solanine concentration-time curves (both after administration of mashed potatoes and test solution) because serum samples could not be quantified up to 72 hours. Calculation of  $AUC_{0-\infty}$  from these curves, resulted in less reliable data because the extrapolation of the area under the curve from  $t_{last}$  to infinity is less reliable.

Regarding  $\alpha$ -chaconine, serum  $\alpha$ -chaconine concentrations could be measured long enough in all subjects who received mashed potatoes, resulting in a reliable extrapolation of the area under the curve from  $t_{last}$  to infinity and thus in reliable data for  $AUC_{0-\infty}$ .

After administration of test solution terminal elimination phase was observed in half of the subjects (both subjects of GA dose 0.50 mg/kg body weight and one subject of GA dose 0.70 mg/kg body weight). In these subjects  $AUC_{0-\infty}$  could be determined adequately. In the remaining subjects,  $AUC_{0-\infty}$  is less reliable.

Besides the elimination phase, two other factors influenced the reliability of the data:

- 1) vomiting of subject 14: the data of subject 14 (for both a solanine and a chaconine) may be too low due to loss of part of the GA dose by vomiting.
- 2) the presence of ghost peaks in the HPLC-chromatograms of the serum samples from test period 3 (mashed potatoes, GA: 1.10 mg/kg body weight) and 4 (mashed potatoes, GA: 1.25 mg/kg body weight and test solution GA: 0.50 mg/kg body weight). The most pronounced effect on  $AUC_{0-\infty}$  on quantification of the ghost peak as either  $\alpha$ -solanine or  $\alpha$ -chaconine was observed in both subjects who received mashed potatoes (GA: 1.25 mg/kg body weight) (see table 3.4A and 3.5 A).

To compare the AUC values of the different test periods it was decided to calculate  $AUC_{0-24h}$  ( $AUC_{0-24h}$  could be determined adequately from the concentration time curve for all of the subjects and for both  $\alpha$ -solanine and  $\alpha$ -chaconine) (see table 3.4 and 3.5).

After administration of mashed potatoes (GA dose 0.95, 1.10 and 1.25 mg/kg body weight),  $AUC_{0-24h}$  of  $\alpha$ -solanine and  $\alpha$ -chaconine were almost within the same range (respectively, 49-149 ng\*h/ml and 45-143 ng\*h/ml) (ghost peak and subject 14 excluded). After administration of test solution (0.30, 0.50 and 0.70 mg/kg body weight),  $AUC_{0-24h}$  of  $\alpha$ -solanine ranged between 35 and 74 ng\*h/ml (ghost peak excluded). A wider range was obtained for  $AUC_{0-24h}$  of  $\alpha$ -chaconine: 41-106 ng\*h/ml (ghost peak excluded).

In general, internal exposure of a compound is expected to increase with increasing dose. After administration of mashed potatoes, this is not observed if all of the three doses are included: the mid dose resulted in the lowest (average)  $AUC_{0-24h}$  values for both  $\alpha$ -solanine and  $\alpha$ -chaconine. If the mid dose is excluded an increase in internal exposure is observed with increasing dose. Regarding test solution, an increase in (average)  $AUC_{0-24h}$  values with increasing dose was observed for  $\alpha$ -chaconine. For  $\alpha$ -solanine, an increase in (average)  $AUC_{0-24h}$  values was observed with a GA dosage increase from 0.30 mg/kg body weight to 0.50 mg/kg. The mid and highest GA dose resulted in comparable (average)  $AUC_{0-24h}$  values. However, these conclusions are not very reliable due to the few number of subjects per dose group and the variation in internal exposure between subjects of the same dose group.

### 3.4.4 $F_{rel}$ of mashed potatoes versus test solution

Relative bioavailability of  $\alpha$ -solanine and  $\alpha$ -chaconine from mashed potatoes compared to test solution was calculated in order to gain insight into the effect of a potato matrix on the bioavailability of GAs. The relative bioavailability of  $\alpha$ -solanine and  $\alpha$ -chaconine for treatment A (mashed potatoes) was estimated by dividing the  $AUC_{0-\infty}$  of treatment A by the  $AUC_{0-\infty}$  of treatment B (test solution), with correction for the administered dose; as explained in 2.9. In addition,  $F_{rel}$  has been calculated by using  $AUC_{0-24h}$  of both treatments as explained above. Calculations of the relative bioavailability of  $\alpha$ -solanine and  $\alpha$ -chaconine from mashed potatoes compared to the test solution, *based on the data of the present pilot study*, should be interpreted as provisional.

The data of  $F_{rel}$  of  $\alpha$ -solanine are presented in table 3.6A, those of  $\alpha$ -chaconine in table 3.6B. It can be observed that per GA dose of mashed potatoes  $F_{rel}$  of  $\alpha$ -solanine and  $\alpha$ -chaconine increased with increasing GA-dose of test solution.  $F_{rel}$  of  $\alpha$ -solanine ranged between 0.5 and 1.9 (using data of  $AUC_{0-24h}$ ).  $F_{rel}$  of  $\alpha$ -chaconine ranged between 0.5 and 1.1 (using  $AUC_{0-24h}$ ). These results can be interpreted as the bioavailability of  $\alpha$ -solanine from mashed potatoes is 0.5-1.9 times the bioavailability of  $\alpha$ -solanine from a test solution and the bioavailability of  $\alpha$ -chaconine from mashed potatoes is 0.5-1.1 times the bioavailability of  $\alpha$ -chaconine from a test solution. Larger ranges were observed for  $F_{rel}$  based on  $AUC_{0-\infty}$ :  $F_{rel}$  of  $\alpha$ -solanine ranged between 0.5 and 2.3 and  $F_{rel}$  of  $\alpha$ -chaconine ranged between 0.5 and 1.4.

Table 3.6A. Relative bioavailabilities of  $\alpha$ -solanine

mashed potatoes/ test solution	0.30 mg/kg BW		0.50 mg/kg BW		0.70 mg/kg BW	
	AUC <sub>0-24h</sub>	AUC <sub>0-∞</sub>	AUC <sub>0-24h</sub>	AUC <sub>0-∞</sub>	AUC <sub>0-24h</sub>	AUC <sub>0-∞</sub>
0.95 mg/kg BW*	1.0	0.7	1.4	1.3	1.9	2.3
1.10 mg/kg BW	0.5	0.5	0.7	0.9	1.0	1.6
1.25 mg/kg BW	0.8	0.7	1.2	1.3	1.6	2.2

\*BW= body weight

Table 3.6 B. Relative bioavailabilities of  $\alpha$ -chaconine

mashed potatoes/ test solution	0.30 mg/kg BW		0.50 mg/kg BW		0.70 mg/kg BW	
	AUC <sub>0-24h</sub>	AUC <sub>0-∞</sub>	AUC <sub>0-24h</sub>	AUC <sub>0-∞</sub>	AUC <sub>0-24h</sub>	AUC <sub>0-∞</sub>
0.95 mg/kg BW*	0.8	0.8	1.1	0.9	1.1	1.4
1.10 mg/kg BW	0.5	0.5	0.6	0.6	0.7	0.9
1.25 mg/kg BW	0.7	0.8	1.0	0.8	1.0	1.3

\*BW= body weight

### 3.5 Adverse/toxic effects

#### 3.5.1 Adverse/toxic effects after administration of mashed potatoes

Table 3.7 shows the adverse effects observed after consumption of mashed potatoes. Subject 17, 21 and 03 experienced some mild adverse effects not clearly related to the consumption of the mashed potatoes. Subject 14 (GA dose: 1.25 mg/kg body weight) however, became nauseous and started vomiting about 4 hours after having consumed 452 g of mashed potatoes. The effects were intermittent (3 times) and of mild intensity ( $\pm$  200, 300 and 200 ml vomit). The effects had cleared up completely about 12 hours post dosing. No change in vital signs were observed. No positive correlation between these symptoms and concentrations of GAs in the serum samples was apparent.  $C_{max}$  and AUC<sub>0-24h</sub> of both GAs were lower than those of subject 09 (GA dose: 1.25 mg/kg body weight) who received almost the same amount of mashed potatoes (440 g) and experienced no adverse effects (see table 3.4A and 3.5A). Therefore, the effects might have been mediated by a local direct effect on the stomach rather than by systemic interactions. These adverse effects may be related to either the GAs or the relatively large amount of mashed potatoes (452 g) consumed on an empty stomach.

All blood cholinesterase concentrations revealed to be within or near the normal range (see appendix 5). Thus, no inhibition of acetylcholinesterase in blood was observed. Furthermore, all vital sign recordings were in the normal range.

None of the participants whom were administered mashed potatoes reported a bitterness of taste. The highest amount of GA in the form of mashed potatoes was administered to subject 22, namely 90 mg (see table 3.1).

*Table 3.7 Adverse effects observed after the consumption of mashed potatoes*

GA-dose (mg/kg BW*)	subject	adverse effects	time post-dosing	duration	intensity	GA related?
0.80	17	Oedema of the eye lids Urticaria of the neck	2nd day	Ongoing upon discharge	Mild	No, due to facial night cream
	21	Headache	12 hours	NR**	Mild	No
0.95	03	Common cold	3rd day	NR	Mild	No
1.25	14	Nausea and vomiting	3:45 hours	Cleared, 11:50 hrs post- dosing	Mild	Possibly

\* BW= body weight

\*\*NR = not recorded

### **3.5.2 Adverse/toxic effects after administration of test solution**

After administration of the test solution no adverse effects did occur. All blood cholinesterase concentrations revealed to be within or near the normal range (see appendix 5). Thus, no inhibition of acetylcholinesterase in blood was observed. Furthermore, all vital sign recordings were in the normal range.

None of the participants whom were administered the test solution reported a bitterness of taste. The highest amount of GA in the form of a test solution was administered to subject 05, namely 50 mg (see table 3.2).



## 4. Discussion

The objective of the present study was to find an optimal sampling scheme and GA dose for a subsequent study on the pharmacokinetics of orally administered GAs in humans.

### 4.1 Optimal blood sampling scheme

As far as the sampling scheme after administration of mashed potatoes and test solution concerns, the final sampling schemes used in the pilot study resulted in time points from which the concentration-time profiles of both  $\alpha$ -solanine and  $\alpha$ -chaconine could be described sufficiently to calculate basic pharmacokinetic parameters.

Three comments can be made on the sampling schemes:

- 1) The sampling scheme following administration of test solution contained two sample points more (0.5 and 1.5 hours post dosing) in comparison to the sampling scheme for mashed potatoes. These sample points were included to obtain a good profile of the peak of the serum GA concentration-time curve (this peak was expected earlier than after administration of mashed potatoes). However, from the results it can be concluded that  $C_{\max}$  after administration of test solution was reached relatively late (4-6 hours post dosing for both GAs) for a solution (generally  $C_{\max}$  has been reached 1-2 hours post dosing). Therefore, the two extra datapoints are not strictly necessary and can be omitted in the final study.
- 2) The sampling scheme after administration of mashed potatoes can be improved by including a sample point at 9 hours post dosing. This is recommended because in one subject of the pilot study  $C_{\max}$  of both GAs was reached rather late: at 8 hours post dosing (subject 14 excluded). However, in this subject  $C_{\max}$  might actually have been reached somewhere between 8 and 10 hours. In order to determine  $C_{\max}$  more precisely in subjects where  $C_{\max}$  of GAs is reached late, it is recommended to include time point 9 hours post dosing in the sampling scheme for mashed potatoes.
- 3) To improve the accuracy of measurements in the tail of the serum concentration-time curve (especially for  $\alpha$ -solanine) three extra samplings should be performed in this period of time, namely 60, 64, and 68 hours postdose.

#### *Recommended sampling schemes for full study*

Mashed potatoes:

$t = -1, -0.5, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, 20, 24, 32, 48, 56, 60, 64, 68, 72$ , and 96 hours, regarding the time point of mashed potato administration as time point zero.

Test solution:

$t = -1, -0.5, 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 20, 24, 32, 48, 56, 60, 64, 68, 72$ , and 96 hours, regarding the time point of test solution administration as time point zero.

## 4.2 Optimal GA dose

To select the optimal GA dose for mashed potatoes and test solution both adverse/toxic effects and the pharmacokinetic results of the pilot study were taken into account.

### *Optimal dose for mashed potatoes*

Administration of mashed potatoes with GA dose 1.25 mg/kg body weight resulted in one of the two subjects in adverse effects that may be related to either the GA dose or to the large amount of mashed potatoes on an empty stomach. In the study of Hellenäs et al. all except one of the seven volunteers reported signs of adverse effects, consisting mainly of light to severe nausea, in one case in combination with diarrhoea [37]. In most instances the symptoms started within the first 30 minutes after the meal and lasted for 3-4 h. No positive correlation between the severity and concentrations of GAs in the serum samples was apparent. Hellenäs et al. concluded that the symptoms might have been mediated by local direct effects on the intestines rather than by systemic interactions. The adverse effects reported by Hellenäs et al. were observed at a total GA-dose of 1.0 mg/kg body weight. In the pilot study no adverse effects were observed after administration of GA doses 0.95 and 1.10 mg/kg body weight. This may be due to the proportion of  $\alpha$ -solanine and  $\alpha$ -chaconine, which differed between both studies. To exclude possible adverse effects caused by the large amount of mashed potatoes, it is recommended to limit the actual amount of mashed potatoes below 350-400 g. As a consequence the maximum GA dose should be  $\leq 1.0$  mg/kg body weight.

From the results of the pilot study it can be concluded that mashed potatoes containing a GA dose  $\geq 0.95$  and  $\leq 1.00$  mg/kg body weight is optimal for the full study. Within this dose range, pharmacokinetics of  $\alpha$ -chaconine can be determined adequately; regarding  $\alpha$ -solanine, at least  $C_{\max}$  and  $t_{\max}$  can be determined. Depending on the further optimisation of the analytical method in serum (see 4.3), the other pharmacokinetic parameters of  $\alpha$ -solanine can probably be determined.

### *Optimal GA dose for test solution*

After administration of test solution (GA dose: 0.3, 0.5, and 0.7 mg/kg body weight) no adverse effects were observed in the pilot study. Furthermore, two of the basic pharmacokinetics of  $\alpha$ -chaconine ( $C_{\max}$  and  $t_{\max}$ ) could be determined adequately for each dose.  $AUC_{0-\infty}$  and  $t_{1/2}$  could be determined adequately in half of the subjects (both subjects of GA dose 0.50 mg/kg body weight and one subject of GA dose 0.70 mg/kg body weight). Regarding  $\alpha$ -solanine,  $C_{\max}$  and  $t_{\max}$  could be determined adequately for each GA dose but  $AUC_{0-\infty}$  and  $t_{1/2}$ , could not. In none of the subjects serum GA concentration time profiles could be followed long enough in time (up to approximately 72 h) to determine  $AUC_{0-\infty}$  and  $t_{1/2}$  of  $\alpha$ -solanine adequately.

From the results of the pilot study it can be concluded that the GA dose range doesn't include an optimal dose to study the basic pharmacokinetics of  $\alpha$ -solanine and  $\alpha$ -chaconine. Since no adverse effects were observed and internal exposure is (in general) lower than after administration of mashed potatoes a higher dose is recommended for the test solution. Based

on the results of the pilot study, it can be expected that a GA dose of 0.90 mg/kg body weight will be appropriate to study the basic pharmacokinetics of  $\alpha$ -chaconine and  $\alpha$ -solanine in all subjects without the occurrence of adverse effects. Regarding  $\alpha$ -chaconine, it is expected that all pharmacokinetic parameters can be determined adequately at this dose. Regarding  $\alpha$ -solanine, at least  $C_{\max}$  and  $t_{\max}$  can be determined at this dose. Depending on the further optimisation of the analytical method in serum (see 4.3), the other pharmacokinetic parameters of  $\alpha$ -solanine can probably be determined. Higher doses are not recommended because adverse effects at these doses can not be excluded.

#### *Recommended GA dose for full study*

Mashed potatoes:

Pharmacokinetics of  $\alpha$ -solanine ( $C_{\max}$  and  $t_{\max}$ )\*: GA dose  $\geq 0.95$  and  $\leq 1.0$  mg/kg BW

Pharmacokinetics of  $\alpha$ -chaconine: GA dose  $\geq 0.95$  and  $\leq 1.0$  mg/kg BW

Test solution:

Pharmacokinetics of  $\alpha$ -solanine\*\*: GA dose 0.90 mg/kg BW

Pharmacokinetics of  $\alpha$ -chaconine: GA dose 0.90 mg/kg BW

\*no suitable dose range for determination of  $AUC_{0-\infty}$  and  $t_{1/2}$  of  $\alpha$ -solanine available.

\*\* this dose may not be suitable to determine  $AUC_{0-\infty}$  and  $t_{1/2}$  of  $\alpha$ -solanine adequately.

BW= body weight

### **4.3 Additional considerations**

In addition to the recommendations on sampling scheme and GA doses, some additional suggestions for the full study can be given:

- In the pilot study a rather large interindividual variation on pharmacokinetics was observed. This was also observed in a pilot study on pharmacokinetics reported by Hellenäs et al. [37]. In the study of Hellenäs et al. maximum concentrations of  $\alpha$ -solanine and  $\alpha$ -chaconine were achieved 4-6 hours and 4-8 hours post dosing, respectively; whereas  $C_{\max}$  ranged between 3.9 and 11.3 ng/ml for  $\alpha$ -solanine and 6.2 and 21.4 ng/ml for  $\alpha$ -chaconine. To get insight into the interindividual variation it is recommended to include approximately 12 subjects per treatment and per dose group in the full study.
- In order to gain insight into the effect of a potato matrix on the bioavailability of GAs, relative bioavailability of  $\alpha$ -solanine and  $\alpha$ -chaconine from mashed potatoes compared to test solution should be calculated in the full study. In the full study, adequate determination of  $F_{\text{rel}}$  of  $\alpha$ -chaconine using values of  $AUC_{0-\infty}$  is expected to be possible. For calculation of  $F_{\text{rel}}$  of  $\alpha$ -solanine, it is recommended to use values of  $AUC_{0-24\text{h}}$  (as demonstrated in the pilot study) if values of  $AUC_{0-\infty}$  are not available. Data of a larger number of individuals (say 12) administered the same dose are needed to calculate the  $F_{\text{rel}}$  more accurately than in the pilot study. Furthermore, the proposed dose for the solution

(0.90 mg/kg body weight) will be more in line with the proposed dose for mashed potatoes (0.95 – 1.00 mg/kg body weight).

- At 24 hours post dosing,  $\alpha$ -solanine and  $\alpha$ -chaconine concentrations are still present in serum in the pilot study (see figure 3.2 and 3.3). This may indicate that daily consumption of mashed potatoes results in accumulation of GAs. If the effect of daily consumption of mashed potatoes on GA and solanidine levels in the human body needs to be studied, it is advisable to include a multiple dose (2 doses) regimen of mashed potatoes in the full study.
- It is recommended to include parameters to monitor adverse/toxic effects of GAs in the full study. The same parameters of the pilot study can be used (vital signs, observation of adverse events and measurement of blood cholinesterase levels).
- The problems with the low, undetectable concentrations of serum  $\alpha$ -solanine may be solved by collecting more blood at each sample point. To study the pharmacokinetics of GAs, 5 ml blood was collected at each sample time point in the pilot study. (In total approximately 150 ml blood per subject was collected). From each blood sample, 2 ml of serum was obtained. This was subsequently analysed for GAs. It is recommended to find out whether instead of 2 ml, 4 ml of serum can be applied for the analytical method. If this optimisation of the method is possible, blood samples of 10 ml per sample time point should be collected in the full study. This may result in serum  $\alpha$ -solanine concentration profiles up to 72 hours and thus, adequate determination of  $AUC_{0-\infty}$  and  $t_{1/2}$ .
- Furthermore, it is recommended to investigate whether the occurrence of the ghost peak, which was observed in the HPLC profile, was not due to an analytical failure to minimise the chance of occurrence of the ghost peak during the full study.
- It is important to investigate the pharmacokinetics of solanidine in the full study, since the site of metabolism of GAs is not clear and the contribution of solanidine to toxic effects of GAs is unknown. Unfortunately, it was not possible to determine solanidine in this pilot study due to analytical problems. It is recommended to optimise the analytical method further, allowing determination of serum solanidine concentrations in the full study.

## 5. Conclusions

Data of the present pilot study indicate that:

- **Both GAs** ( $\alpha$ -solanine and  $\alpha$ -chaconine) seem to have the **potential to accumulate** in the human body, given the used concentration of 199 mg per kg potatoes. Total clearance of both compounds takes place far beyond the 24 hr time point post dosing. This probably holds true for the majority of the general population.
- **Interindividual variability** in the bioavailability of GAs is **potentially large**. That means that a proportion of the general population may be more vulnerable to GAs than others. A study with a larger number of subjects is necessary to evaluate this issue.
- **Taste of bitterness** doesn't seem to be a **reliable indicator for GA concentration**, given the use of the relatively high concentration of 199 mg per kg potatoes. That means that it is likely that higher GA concentrations can be consumed without being noticed.
- **Gastrointestinal adverse effects** (such as nausea and vomiting) are **possibly related to consumption** of potatoes or potato product with relatively high concentrations of GAs. In the present study, one subject whom was administered the highest dose of mashed potatoes experienced these effects.
- **Systemic adverse effects** (such as low blood pressure, rapid respiration) were **not observed** after the present single dose administrations. However, further investigation (including measurement of solanidine) is necessary to gain more insight the metabolism of GAs in relation to the occurrence of these adverse effects.

## References

1. Desfosses M: Extrait d'une letter de M. Desfosses, pharmacien, à Besançon, à M. Roubiquet. J Pharm 1820;6:374-376.
2. Schreiber K: in Steroid alkaloids: the *Solanum* group. Manske, RHF, ed. The alkaloids: chemistry and physiology. Vol. 10. Academic Press, New York, 1968.
3. Baup M: Extrait d'une letter de M. Baup aux redacteurs sur plusieurs nouvelles substances. Ann Chim Phys 1826;31:108-109.
4. Kuhn R, Löw I: Die Konstitution des Solanins. Angew Chem 1954;66:639-640.
5. Kuhn R, Löw I, Trischmann H: Die Konstitution des Solanins. Chem Ber 1955;88:1492-1507.
6. Kuhn R, Löw I, Trischmann H: Die Konstitution des  $\alpha$ -Chaconins. Chem Ber 1955;88:1690-1693.
7. Prelog V, Jeger O: Steroid alkaloids: the *Solanum* group. In: The Alkaloids, vol VII, RHF Manske (Ed.). Academic Press, New York, 1960, pp. 343-361.
8. Schreiber K: Steroid alkaloids: the *Solanum* group. In: The Alkaloids, vol X, RHF Manske (Ed.). Academic Press, New York, 1968, pp. 1-192.
9. Ripperger H, Schreiber K: *Solanum* steroid alkaloids: the *Solanum* group. In: The Alkaloids, vol XIX, RGA Rodrigo (Ed.). Academic Press, New York, 1968, pp. 81-192.
10. Van Gelder WMJ: Determination of the total C27-steroidal alkaloid composition of *Solanum* species by high resolution SGA chromatography. J Chromatogr 1985;331:285-293.
11. Jadhav SJ, Sharma RP, Salunkhe DK: Naturally occurring toxic alkaloids in foods. CRC Critical Reviews in Toxicology 1981;9:21-104.
12. Johnson H, Hellenäs KE: Glycoalkaloids in potato. Var Föda 1983; 35:299-314.
13. Zitnak A: The occurrence and distribution of free alkaloid solanidine in Netted Gem potatoes. Can J Biochem Physiol 1961;39:1257-1265.
14. Wood FA, Young DA: TGA in potatoes. Can Dep Agric Publ 1974;1533.
15. Verbist JF, Monnet R: A propos de la teneur en solanine des petits tubercules nouveaux de pomme de terre (*Solanum tuberosum* L). Potato Res 1979;22:239-244.
16. Friedman M, Mc Donald GM: Potato glycoalkaloids: chemistry, analysis, safety and plant physiology. Critical Reviews in Plant Sciences 1997; 16:55-132
17. Krasowski MD, McGehee DS, Moss J: Natural inhibitors of cholinesterase's: implications for adverse drug reactions. Can J Anaesth 1997;44: 525-534.
18. Blankemeyer JT, Stringer BK, Rayburn JR, Bantle JA, Friedman M: Effect of potato glycoalkaloids  $\alpha$ -chaconine and  $\alpha$ -solanine in membrane potential of frog embryos. J Agric Food Chem 1992;40:2022-2026.
19. Friedman M, Rayburn JR, Bantle JA: Structural relationships and developmental toxicity of *Solanum* alkaloids in the frog embryo teratogenesis assay. Xenopus J Agric Food Chem 1992;40:1617-1624.
20. Rayburn JR, Bantle JA, Friedman M: Role of carbohydrate side chains of potato glycoalkaloids in developmental toxicity. J Agric Food Chem 1994;42:1511-1515.
21. British Medical Journal: Solanine Poisoning. Dec 8, 1979, 1458-1459.
22. Sinden SL: Potato glycoalkaloids. Acta Hort 1987;207:41-47.
23. McMillan M, Thompson JC: An outbreak of suspected solanine poisoning in schoolboys: examination of criteria of solanine poisoning. Quarterly Journal of Medicine 1979;48:227-243.

24. Morris SC and Lee TH: The toxicity and teratogenicity of Solanaceae glycoalkaloids particularly those of the potato (*Solanum tuberosum*): a review. *Food Technol Aust* 1984;36:118-124.
25. Bömer A, Mattis H: Der Solaniningehalt der Kartoffeln. *Z. Unters Nahr-Genussm* 1924;47:97-127
26. Slanina P: Solanine (glycoalkaloids) in potatoes: toxicological evaluation. *Food and Chemical Toxicology* 1990;28:759-761.
27. Ross H, Pasemann P, Nitsche W: Der Glykoalkaloidgehalt von Kartoffelsorten in seiner Abhängigkeit von Anbauort und -jahr und seiner Beziehung zum Geschmack. *Z Pflanzenzuecht* 1978;80:64-79.
28. Slanina P: Assessment of health-risk related to glycoalkaloids ('solanine') in potatoes: A nordic view. Report from the Nordic Working Group on Food Toxicology and Risk Assessment. *Vår Föda* 1990;43:1-15, suppl 1.
29. JECFA: Toxicological evaluation of certain food additives and naturally occurring toxicants. WHO Food Additives Series 1993;30:339-372.
30. IPCS/WHP/ILSI: Steering Committee on Natural Toxins, Report published by IPCS, WHO, Geneva, Switzerland, 1992.
31. Groen K, Pereboom-de Fauw DPKH, Besamusca P, Beekhof PK, Speijers GJA, Derks HJGM: Bioavailability and disposition of <sup>3</sup>H-solanine in rat and hamster. *Xenobiotica* 1993;23:995-1005.
32. Centraal Bureau voor de Statistiek. Statistisch Jaarboek. Voorburg/Heerlen, 1997.
33. Van Gelder WMJ: Solanum alkaloiden: voorkomen in plantaardige voedingsmiddelen, De Ware(n)-Chemicus 1989;19:164-171.
34. Sizer CE, Maga JA, Craven CJ: Total glycoalkaloids in potatoes and potato chips. *J Agric Food Chem* 1980;28:578-579.
35. Sinden SL, Deahl KL, Aulenbach BB: Effect of glycoalkaloids and phenolics on potato flavor. *J Food Sci* 1976;41:520-523.
36. Zitnak A: Steroids and capsaicinoids of Solanaceous food plants. In: *Nightshades and Health*, Eds. Childers NF and Russo GM, Somerset Press, Somerville, NJ, 1977, pp.41-91.
37. Hellenäs K-E, Nyman A, Slanina P, Löf L, Gabrielsson J: Determination of potato glycoalkaloids and their aglycone in blood serum by high-performance liquid chromatography. Application to pharmacokinetic studies in humans. *Journal of Chromatography* 1992; 573:69-78.
38. Nishie K, Gumbmann MR, Keyl AC: Pharmacology of solanine. *Toxicol. Appl. Pharm.* 1971;19:81-92.
39. Claringbold WDB, Few JD, Renwick JH: Kinetics and retention of solanidine in man. *Xenobiotica* 1982;12:293-302.
40. Harvey MH, McMillan M, Morgan MRA, Chan HW-S: Solanidine is present in sera of healthy individuals and in amounts dependent on their dietary potato consumption. *Human Toxicology* 1985;4:187-194.
41. Harvey MH, Morris BA, McMillan M, Marks V: Measurement of potato steroidal alkaloids in human serum and saliva by radioimmunoassay. *Human Toxicology* 1985;4:503-512.
42. The 30<sup>th</sup> Meeting of the Joint FAO/WHO Expert Committee on Food Additives: Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series 1986;21:173-219.

## Appendix 1 Composition and preparation of mashed potatoes

### 5.1.1.1 Composition of potatoes

Potatoes of the variety 'Elkana' have been used for production of potato flakes.

Potato characteristics of these potatoes have been determined by 'Nederlands Instituut voor Koolhydraat Onderzoek' TNO, Groningen:

Dry matter	25.8% m/m
Starch	18.7% m/m
Total sugar	1.51% m/m
Total raw protein	2.83% m/m
Glycoalkaloids:	310 mg/kg unpeeled potatoes
(180 mg $\alpha$ -solanine, 110 mg $\alpha$ -chaconine and 20 mg $\beta$ -chaconine/kg)	

Potatoes have been steam peeled, boiled, mashed and dried resulting in potato flakes by Agrotechnical Research Institute (ATO-DLO), Wageningen. The potato flakes have been canned under nitrogen atmosphere and stored at 4°C until use.

### *Recipe of mashed potatoes:*

Add 100 g potato flakes to 480 ml hot water (90°C).

Stir until the potato flakes have been hydrated.

Add some salt, pepper [peper] and paprika.

### *Composition of mashed potatoes (per kg):*

828	ml	tap water
171	g	potato flakes containing GAs*
1.1	g	Dimodan**
0.08	g	sulphite (Na <sub>2</sub> S <sub>2</sub> O <sub>5</sub> )
some salt, pepper and paprika		

\* GAs: approximately 101 mg  $\alpha$ -solanine and approximately 98 mg  $\alpha$ -chaconine per kg mashed potatoes.

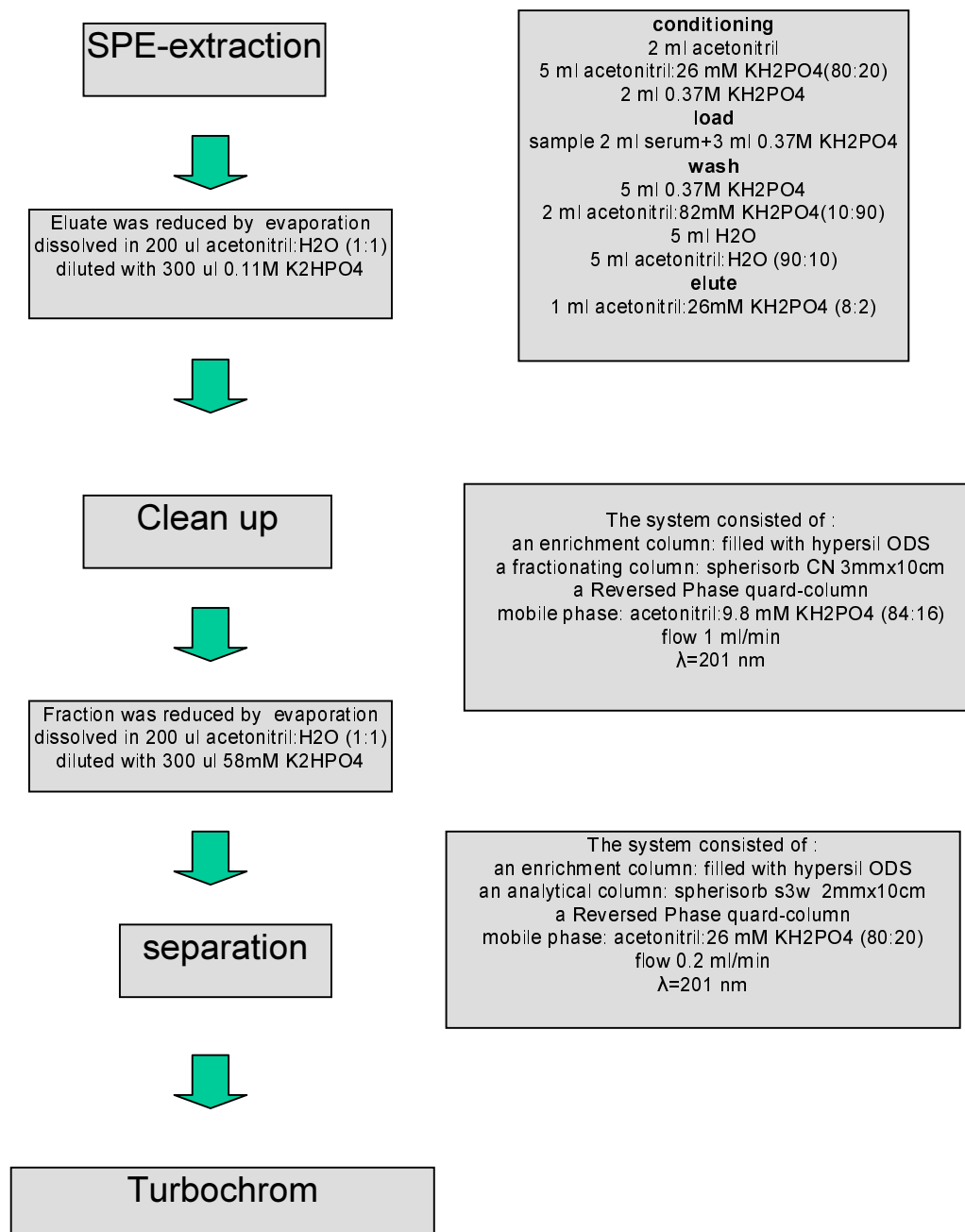
\*\* Dimodan is an emulsifier which has been added during the production of the potato flakes. The quantity of Dimodan present in mashed potatoes is within the normal usage range for potato products: 0.9% Dimodan of starch (1 kg mashed potatoes contains 124 g starch).

\*\*\* Sulphite (54.0 mg SO<sub>2</sub> equivalents/kg mashed potatoes) has been added during the production of the potato flakes to improve the keeping qualities of the potato flakes.

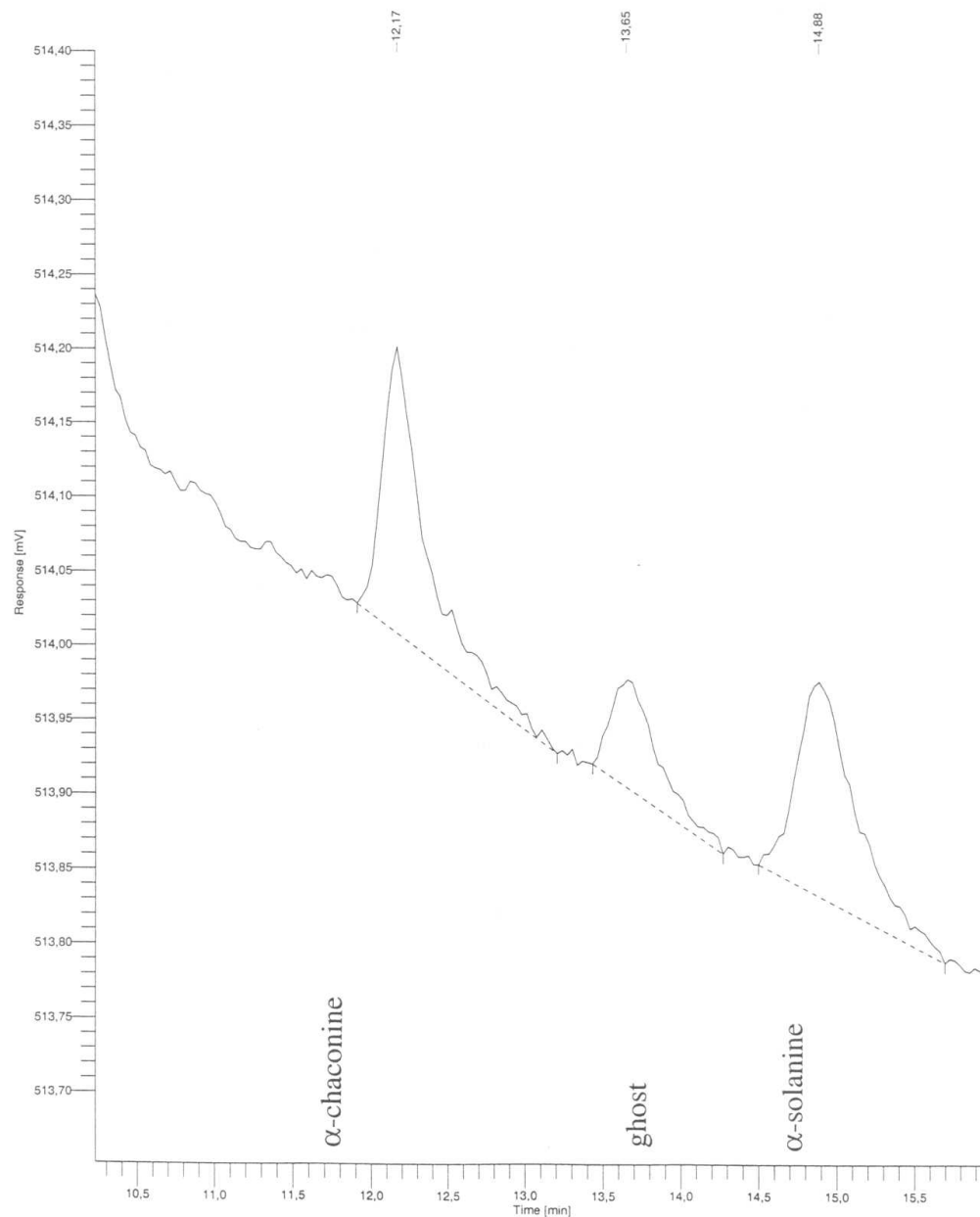
The acceptable daily intake (ADI) of sulphite is 0-0.7 mg/kg body weight for SO<sub>2</sub> en SO<sub>2</sub> equivalents [42]. In subjects receiving 0.8 mg GAs/kg mashed potatoes the intake of sulphite is within the acceptable range: 0.2 mg/kg body weight for SO<sub>2</sub> and SO<sub>2</sub> equivalents.



## Appendix 2 Schematic overview of the analysis of $\alpha$ -solanine and $\alpha$ -chaconine in human serum



### Appendix 3 Example of HPLC chromatogram of $\alpha$ -solanine and $\alpha$ -chaconine including a ghost peak



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## **Appendix 5 Clinical report**

Clinical report

**Clinical pilot study on the  
bioavailability of glycoalkaloids from potato**

March 16<sup>th</sup>, 2001

Earl Johannis, Tjeert Mensinga

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**ABBREVIATIONS**

ALAT	Alanine aminotransferase
ASAT	Aspartate aminotransferase
approx.	Approximately
ARO	Laboratory for Residue Analysis (Laboratorium voor Analytisch Residu-onderzoek)
AUC	Area under the curve
BMI	Body mass index
bw	Body weight
CAS	Chemical Abstracts Service
CL	Clearance
C <sub>max</sub>	Maximum concentration measured
CPK	Creatinine Phosphokinase
CRF	Case Report Form
ECG	Electro Cardiogram
ESR	Erythrocyte Sedimentation Rate
GA	Glycoalkaloid(s)
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GP	General practitioner
h	Hour(s)
Hb	Haemoglobin
HPLC	High Performance Liquid Chromatography
Ht	Haematocrit
iv, i.v.	Intravenous
l, L	Litre(s)
LBO	Laboratory for Exposure Assessment (Laboratorium voor Blootstellingsonderzoek)
MW	Molecular weight
NOEL	No observed effect level
NVIC	National Poisons Control Centre (Nationaal Vergiftigingen Informatie Centrum)
po	per os
RIVM	National Institute of Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu)
SEM	Standard Error of the Mean
SOP	Standard Operating Procedure
t <sub>max</sub>	Time point corresponding to C <sub>max</sub>
t <sub>½</sub>	Terminal half life
UMC Utrecht	University Medical Centre Utrecht
UTN	Unique Trial Number
VWS	Ministry of Public Health, Welfare, and Sports (Ministerie van Volksgezondheid, Welzijn en Sport)
γGT	Gamma Glutamyl transpeptidase

# 1. Introduction

Glycoalkaloids are natural toxins found in the potato plant (*Solanum tuberosum*) and other solanaceous plants such as the tomato plant (*S.lycopersicum*), egg plant (*S.melongena*), and wild and garden nightshade (*S.dulcamara* and *S.nigrum*). These glycoalkaloids, *abbreviated as GA for the remainder of this report*, are involved in plant defence against pathogenic organisms and insects and are also potentially toxic for animals and humans (1-3).

The initial GA levels are genetically determined and vary greatly between the different potato cultivars. The highest GA levels in potato are present in the foliage, blossoms and sprouts. Within the tuber the levels are greatest in the layer just beneath the peel and the "eyes". In commercial potato cultivars the primary GA's are  $\alpha$ -solanine and  $\alpha$ -chaconine which are glycosides of the same steroidal alkaloid solanidine. Together these two comprise approx. 95% of total potato GA level (4-9).

GA in potato can cause a bitter taste when present in concentrations above 200 mg/kg of fresh weight (10). In commercial potato the levels of GA are generally low, on average 20-80 mg/kg fresh weight. Several factors such as poor growing conditions, exposure to light (green potato), damage during mechanical harvesting, inadequate storage conditions and industrial processing can lead to a significant increase in GA levels. Small and young tubers may have GA levels of up to 800 mg/kg fresh potato (2;11). GA will not be destroyed by processes such as boiling, baking, frying or drying at high temperatures (12;13). Peeling of potato can reduce the levels in the tuber by 60 to 96%. Due to incomplete peeling of the tubers and dehydration caused by frying, French fries or potato chips may contain GA levels of up to 720 mg/kg (11). Thus, it is not unlikely that the consumption of these products could lead to ingestion of possibly toxic levels of GA.

GA's are toxic in two respects: by cellular membrane disruption (affecting the gastrointestinal tract) and by cholinesterase inhibition (affecting the central nervous system). There is a significant species difference in GA toxicity (1;3), probably due to differences in absorption from the gastrointestinal tract, in faecal and urinary excretion as well as the hydrolysis of  $\alpha$ -solanine and  $\alpha$ -chaconine to solanidine (14;15).

Based on empirical data derived from acute human poisoning, it is generally accepted that potatoes with GA levels of up to 200 mg/kg fresh weight are safe for human consumption (16). From the data of the average yearly consumption of potato, the average daily intake of potato in the Netherlands is estimated to be approx. 230 g (17). With an average GA level of 20-80 mg/kg fresh weight in commercial potato, the individual daily GA consumption would be 18.4 mg or less. However, if potatoes would contain higher GA concentrations, e.g. 200 or 800 mg/kg fresh weight, than the individual daily GA consumption would be 46 or 184 mg.

The toxic dose of GA for man is estimated to be 2-5 mg/kg body weight (140-350 mg for a person of 70 kg) and the lethal dose is 3-6 mg/kg body weight (210-420 mg for a person of 70 kg). Clinical symptoms observed in man after a latency period of 2-20 hr are gastrointestinal disturbance such as nausea, vomiting, diarrhoea and severe abdominal pain. More severe cases develop neurological symptoms such as drowsiness, apathy, confusion, agitation and hallucinations. Death may be caused by haemodynamic instability and cardiac arrhythmias (18-21).

For a proper risk assessment of GA toxicity to man, it is crucial to gain information on the bioavailability of GA from potato in humans. This would allow us to determine a safe level of intake and formulate a substantiated recommendation of acceptable GA levels in potato.

The limited information on the toxicity of GA's in potato in relation to the oral dose requires the performance of a pilot dose-finding study. The results of the pilot study will be used to determine an optimal dosing and sampling regime for a bioavailability study to be performed subsequently, with a larger number of subjects for a given dose.

## 2. Materials and methods

### 2.1 Materials

#### 2.1.1 Study population

Volunteers for the present pilot study were recruited through direct mailing to individuals whom had participated in previous studies of the National Poison Control Centre (NVIC) in Utrecht or had indicated their willingness to do so. Announcements were also posted on the bulletin boards throughout the buildings of Utrecht University and in the Utrecht University Newspaper.

One month to a week before the study the volunteers received both written and verbal information about the purpose, design, potential risks and benefits of the study. A written informed consent (Appendix 1, on p. 64) was obtained before proceeding with the medical screening. Volunteers were enrolled into the study based on their compliance to the inclusion criteria (Appendix 2, on p. 65), their medical history, physical examination and results of the ECG, haematological, biochemical and urine laboratory analysis. If any of the laboratory results showed a significant deviation from the normal value, a repeat analysis was performed. Only if no clinically significant abnormalities were found were the volunteers included into the study. With the volunteers approval their General Practitioner was informed of their enrolment into the study and requested to inform us if he/she is familiar with any reason to exclude the volunteer from study participation. After the study the volunteers were subjected to a second medical examination before discharge.

#### 2.1.2 Clinical Research Unit and co-operating Laboratories

A	The Clinical Research Unit of the National Poison Control Centre (NVIC/RIVM, Utrecht), in collaboration with the Intensive Care Unit 1 & Clinical Toxicology (University Medical Centre Utrecht), performed the pilot study
B	The Laboratory for Project Research <i>U-Diagnostics</i> (situated at the University Medical Centre Utrecht) performed the clinical routine analysis (haematology and biochemistry).
C	The Laboratory for Exposure Assessment and Environmental Epidemiology (LBM/RIVM, Bilthoven) performed the measurements of glycoalkaloids in serum.
D	The Laboratory for Residue Analysis (ARO/RIVM, Bilthoven) performed the measurements of glycoalkaloids in mashed potato.

#### 2.1.3 Study compounds

A	The mashed potato containing $\alpha$ -solanine and $\alpha$ -chaconine was freshly prepared from potato flakes supplied by Agrotechnical Research Institute (ATO-DLO, Wageningen). Tinned potato flakes were mixed with hot tap water (90°C) less than an hour before consumption by the volunteers. Appendix 3 (on p. 66) lists the composition and preparation procedures of the administered mashed potatoes. The concentration of GA's (on average 50.8% $\alpha$ -solanine and 49.2% $\alpha$ -chaconine) in mashed potatoes was 199 mg per kg.
B	The GA solution was freshly prepared (less than an hour before consumption) by the Central Pharmacy of the UMC Utrecht. The proper amounts of 50% $\alpha$ -solanine (CAS20562-02-1) and 50% $\alpha$ -chaconine (CAS 20562-03-02), supplied by Fluka, were dissolved in 750 ml of distilled water. The dose-dependent concentrations of glycoalkaloids in the used solutions are listed in Tabel 7 (on p. 61).



## 2.2 Methods

### 2.2.1 Study protocol

The study was carried out in accordance with the RIVM-protocol “Clinical pilot study on the bioavailability of glycoalkaloids from potato” project number: 388802, dated June 11<sup>th</sup>, 1998. The Medical Ethics Committee of the University Medical Centre Utrecht (UMC Utrecht) approved the study protocol on September 04<sup>th</sup>, 1998.

In the above mentioned original study protocol, the dose steps for the GA-solutions were planned from 0.20 to 0.30 to 0.40 and finally to 0.50 mg GA per kg bodyweight. However, after the first two doses, the interim kinetic analysis showed that a dose increase to 0.40 and 0.50 mg GA per kg bw would not be sufficient to yield an appropriate concentration-time curve for kinetic evaluation. The observed plasma GA-concentrations were found to be too low. None of the first four volunteers experienced any significant adverse events. In view of these interim results, the Medical Ethical Review Board of the UMC Utrecht was asked (on March 18<sup>th</sup>, 1999) to review a protocol amendment (Appendix 4, on p. 67), requesting an increase in the third and fourth dose step to respectively 0.50 and 0.70 mg GA per kg bw. This protocol amendment was approved on March 24<sup>th</sup>, 1999.

### 2.2.2 Summary of study procedures

Table 1, presents a schedule of the study procedures used during the present pilot study, irrespective of the actually (individually) administered test product. For each participant, the study was performed over a period of 5 days. Each participant only received one single dose of either mashed potatoes or a GA-solution, and received only one concentration step (see also Table 3, discussed in the next paragraph).

On the evening before administration of the test product, participants have refrained from eating or drinking (with the exception of water) after 23:00 hours (as stated by the participants). The test product was administered on examination day 0 (Table 1, third column). In order to optimize gastric passage, participants were instructed to sit up straight in bed for the first four hours after test product administration. Each of the 19 participants stayed at the Clinical Research Unit for the first 24-hour period post-dosing. Thereafter, they attended the Unit five times at 32, 48, 56, 72, and 96 hours postdose.

Table 1. Study procedures

Study procedures	Check-in	Day 0	Day 1-4	Check-out
Medical history	X	X	X	X
Physical examination	X	X*	X*	X
Vital signs	X	X	X	X
Pregnancy test		X		
Blood sampling				
• for chemical, haematological tests	X			X
• for cholinesterase tests	X	X	X	X
• for bioavailability tests <sup>†</sup>		X	X	
Urine testing	X			
Test product administration		X		

\* If indicated by medical history

† All blood sampled for bioavailability will be separated into a serum fraction and a cellular fraction. glycoalkaloid levels will be determined in the serum fraction

During the first 24-hour period post dosing, frequent blood samplings were performed. For reasons of convenience (for both participants and clinical investigators) an intravenous cannula was inserted in the participant's forearm. In this way samplings of blood were performed at the following time points (Table 2), regarding the time point of test product administration as time point zero.

*Table 2. Blood sampling time points at the first examination day*

Time reference	Blood sampling time points
Predosing	• At 1, and ½ hours (to determine the baseline concentrations)
Postdosing	• At 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 20, and 24 hours • Additionally at ½, and 1½ hours, in case of administration of a GA-solution.

After the first 24-hour post dose time period, the intravenous cannula was removed. Further blood samplings were performed at 32, 48, 56, 72, and 96 hours post dose by venepuncture during the return visits.

### 2.2.3 Summary of the study design

The study was designed as an open study, with a stepwise ascending dose. Seventy-two hours prior to and during the study the volunteers were placed on a diet free from potato or products containing potato constituents (Appendix 5, on 69). Volunteers received one of the following eight treatments, as listed in Table 3.

*Table 3. Study compounds and administered glycoalkaloid concentration*

	Study compound	Glycoalkaloid concentration (mg per kg bodyweight)
A <sub>1</sub>	Mashed potatoes	0.80
A <sub>2</sub>		0.95
A <sub>3</sub>		1.10
A <sub>4</sub>		1.25
B <sub>1</sub>	GA solution	0.20
B <sub>2</sub>		0.30
B <sub>3</sub> <sup>*</sup>		0.50
B <sub>4</sub> <sup>*</sup>		0.70

\* Concentrations after protocol amendment.

The starting dose of 0.80 mg GA/kg bw. in mashed potato was derived from the presently acceptable "safe" non-toxic level of 200 mg GA per kg fresh potato. Since an oral solution may induce adverse symptoms at a lower dose a conservative dose of 0.20 mg GA/kg bw. was chosen to start with. A consumption period of 30 minutes was allowed for both the mashed potato and the oral solution. The volunteers were monitored over a 5-day period, this entailed a 24-hour admission period and 5 return visits. During these visits, the vital signs and adverse events were registered and blood samples for laboratory analysis and bioavailability tests were drawn.

### 2.2.4 Vital signs and Adverse events

Blood pressure and heart rate were monitored with a non-invasive automated blood pressure meter (Passport Monitor of Datascope®). Twelve-lead electrocardiograms were obtained using a Hewlett Packard cardiograph (type 4700-A). During the study any adverse events observed or reported were documented. The volunteers were requested to report all such events whether related to the dosing or not.

### 2.2.5 Laboratory analysis

Blood samples for laboratory analysis and for bioavailability tests were obtained by venepuncture in the fossa cubiti or from an iv. cannula inserted in the forearm. A total of approx. 150 ml of blood was

taken from the volunteers during the study. The following haematological and biochemical parameters were determined (Table 4).

*Table 4. Haematological and biochemical parameters*

	Parameters
Haematology	<ul style="list-style-type: none"> <li>• Hb</li> <li>• Ht</li> <li>• Leukocytes count, total and differential</li> <li>• Thrombocyte count</li> <li>• ESR</li> </ul>
Biochemistry	<ul style="list-style-type: none"> <li>• Sodium</li> <li>• Potassium</li> <li>• Chloride</li> <li>• Urea</li> <li>• Calcium</li> <li>• Creatinine</li> <li>• CPK</li> <li>• Alkaline phosphatase</li> <li>• ASAT</li> <li>• ALAT</li> <li>• <math>\gamma</math>GT</li> <li>• Total proteins</li> <li>• Albumin</li> <li>• Cholinesterase</li> </ul>

The precision of the routine laboratory tests was assured by inter-laboratory surveillance procedures ("ring validation" method). Furthermore, during the pre-study medical screening urine samples were collected for dipstick urine analysis on glucose, protein, blood and leukocytes.

For bioavailability tests, serum samples were obtained after complete precipitation of the cellular fraction (at room temperatures).

## 2.3 Reports and Archiving

The clinical results of this pilot study are documented in a clinical report, written by the clinical investigators of the National Poison Control Centre (NVIC). The final report (including the clinical report), will be written by the researchers of the Laboratory for Exposure Assessment and Environmental Epidemiology (LBM/RIVM). All clinical study documents will be kept on file in the GCP-archives of the National Poison Control Centre in Utrecht.

## 3. RESULTS

### 3.1 Demographic data at time of enrolment

Appendix 6 (on p. 70) summarizes the demographic data of 25 volunteers who applied for participation in the study. A Yes/No statement in the second column indicates whether or not a potential participant was included in the study. They were allocated to a unique trial number (UTN) by order of appointment for the examination of check-in:

- Six of these potential participants (UTN01, UTN02, UTN07, UTN08, UTN13, and UTN16) were excluded from participation for different reasons (further described in §3.2).
- The remaining 19 subjects aged 20-34 were enrolled in the study: 9 males and 10 females.

### 3.2 Examination of check-in

The first check-in examination was performed on February 01<sup>st</sup>, 1999. Each of the above mentioned 25 subjects (willing to participate in the pilot study on the bioavailability of GA's from potato) underwent a medical screening to determine if they met all criteria for inclusion into the study. The medical screening entailed a review of their medical history, physical examination, electrocardiography, blood sampling for routine haematological and biochemical blood testing, and urine sampling for routine urine analysis. To document the decisions to include or exclude potential participants in the study, appendix 7 (on p. 71) summarizes the abnormal findings and/or relevant issues registered during the medical screening.

### 3.3 Observations during the study

#### 3.3.1 Adverse effects

##### 3.3.1.1 *Adverse effects after administration of mashed potatoes*

Eleven volunteers received the GA dose in the form of mashed potato containing 199 mg GA per kg potatoes. In accordance with the original protocol, the GA dose administered increased in four steps from 0.80 to 0.95 to 1.10 and finally to 1.25 mg GA per kg bw. The actual amounts of GA administered per kg bw are listed in Table 5, by UTN number. In the first three dosing steps, 3 volunteers received a dose of mashed potato, while in the final dosing step only 2 volunteers received a dose of mashed potato.

Table 6, shows the adverse effects observed after consumption of mashed potato. Volunteers UTN17, UTN21 and UTN03 experienced some mild adverse effects not clearly related to the consumption of the mashed potato. UTN14 (bodyweight: 72 kg) however, became nauseous and started vomiting about 4 hours after having consumed 452.3 g of mashed potato. The effects were intermittent (3 times) and of mild intensity (approx. 200, 300 and 200 ml vomit). The effects had cleared up completely about 12 hours post dosing. No changes in vital signs were observed. These adverse effects were interpreted as not systemic. The plasma GA concentrations were not particularly high and comparable to those of UTN09 (bodyweight: 70 kg) who received almost the same dose (439.7 g mashed potato) and experienced no adverse effects. Furthermore, UTN22 (bodyweight: 82 kg) received an even higher dose (453.3 g mashed potato) also without any adverse effects. Therefore, the adverse effects experienced by UTN14 were very likely related to the relatively high dose of 452.3 g of mashed potatoes consumed on an empty stomach. However, it cannot be excluded that the observed effects are due to a direct influence of GAs on the gastrointestinal wall (not a systemic but nevertheless a local effect).

*Table 5. Oral dose of glycoalkaloids in mashed potatoes per kg bodyweight*

UTN	Dosing date	GA* dose mg/kg bw	Bodyweight kg	Mashed potato 199mgGA/kg	Total mg GA*
				g	
12	February 15th	0.80	72	289.0	57.60
17			79	317.6	63.20
21			76	305.9	60.80
03	March 01st	0.95	67	319.8	63.65
10			70	334.2	66.50
11			79	377.1	75.05
15	March 15th	1.10	72	398.0	79.20
20			71	392.5	78.10
22			82	453.3	90.20
09	March 29th	1.25	70	439.7	87.50
14			72	452.3	90.00

\* GA = glycoalkaloids

*Table 6. Adverse effects observed after the consumption of mashed potatoes*

UTN	Adverse effects	Time post-dosing	Duration	Intensity	Drug related
17	Oedema of the eye lids Urticaria of the neck	2nd day	Ongoing upon discharge	Mild	No, due to facial night cream
21	Headache	12 hours	NR*	Mild	No
03	Common cold	3rd day	NR	Mild	No
14	Nausea and vomiting	3:45 hours	Cleared, 11:50 hrs post-dosing	Mild	Possibly

\* NR = not recorded

**3.3.1.2 Adverse effects after administration of a glycoalkaloid solution**

In this dose-finding study, eight volunteers received an oral dose of GA in the form of a solution. The GA dose was increased in four steps from 0.20, 0.30, 0.50 and finally 0.70 mg GA per kg bw. During each dose step 2 participants received an oral dose of the GA solution. Table 7 lists the actual amounts of GA administered according to bodyweight. Adverse effects did not occur.

*Table 7. Oral dose of the glycoalkaloids solution per kg bodyweight*

GA* dose mg/kg bw	UTN	Dosing date	Bodyweight kg	Solution		Total mg GA*
				mg GA /ml	ml	
0.20	04	Feb. 16th	66	0.064	206.3	13.20
	06		82		256.3	16.40
0.30	18	March 02nd	66	0.096	206.3	19.80
	19		61		190.6	18.30
0.50	24	March 30th	65	0.160	203.1	32.50
	25		60		187.5	30.00
0.70	05	April 12th	71	0.224	221.9	49.70
	23		62		193.8	43.40

\* GA = glycoalkaloids, Composition: 50%  $\alpha$ -solanine and 50%  $\alpha$ -chaconine

### 3.3.2 Diet adherence

There were no relevant aberrations from the study diet. UTN03 had a portion of potato salad ( $\pm 40$ g) three days prior to dosing, UTN04 had eaten a few chips 15 hours prior to dosing. And UTN05 had eaten some French fries 3 days prior to dosing.

### 3.3.3 Sensory perceptions of bitterness of taste

None of the participants whom were administered mashed potatoes (with a GA concentration of 199 mg per kg potatoes) reported a bitterness of taste. The highest amount of GA in the form of mashed potatoes was administered to UTN22, namely 90.2 mg (in 453.3 g of mashed potatoes).

Also, none of the participants whom were administered the GA-solution potatoes (with a GA concentration of up to 224 mg per litre) reported a bitterness of taste. The highest amount of GA in the form a GA-solution was administered to UTN05, namely 49.7 mg (in 221.9 ml of the GA-solution with the highest GA concentration).

### 3.3.4 Toxicity monitoring

At lower doses, the toxicity of GA in humans mainly concerns gastrointestinal disturbances like vomiting, diarrhoea and abdominal pain. At higher doses, however, the toxicity of GA in humans concern more severe symptoms, with fever, rapid pulse, low blood pressure, rapid respiration, and neurological symptoms. Even several cases of lethal poisoning due to GA exposure have been reported. The GA's  $\alpha$ -solanine and  $\alpha$ -chaconine are known to be inhibitors of acetylcholinesterase. Therefore, cholinesterase levels were measured before the start of the study, during the study and after the study. All observed concentrations revealed to be within or near the normal range (Appendix 8, on p. 74).

## 3.4 Examination of check-out

The medical screening before discharge showed no abnormalities that prevented a discharge from the study. There were no remarks at all for UTN05, UTN09, UTN10, UTN12, UTN14, UTN18, UTN20, UTN21, UTN22, and UTN24. Appendix 9 (on p. 75) summarizes the remarks for the other 9 participants.

## 4. Conclusions

The following conclusions can be derived from the results of the present pilot study:

- Adverse effects: Neither moderate nor severe adverse effects did occur. With the exception of one individual (UTN14), all of the observed mild adverse affects were not directly associated with the administration of glycoalkaloids. In case of UTN14, repeated nausea and vomiting occurred after administration of mashed potatoes. This participant (bw 72 kg) received the highest dose of 1.25 mg GA/kg bw (in total 90 mg GA), by consumption of 452.3 g of mashed potatoes. These adverse effects were likely associated with the consumption of a relatively large quantity of more than 450 g of mashed potato. However, it cannot be excluded that the observed effects are due to a direct influence of GAs on the gastrointestinal wall (not a systemic but nevertheless a local effect). There were no observations of changes in vital signs, and the plasma concentrations of GA were normal.
- Vital signs: All vital sign recordings were in the normal range
- Sensory perceptions: None of the participants reported a bitterness of taste, given the GA concentration of 199 mg/kg in mashed potatoes and given the GA concentration of up to 224 mg/liter in the GA-solution.
- Toxic effects: All cholinesterase concentration levels were normal.

The overall conclusion is, that the clinical results observed during this pilot study do not obstruct continuation of the study with  $\alpha$ -solanine and  $\alpha$ -chaconine in mashed potatoes and oral solutions (given the used concentrations). However, it is recommended to somewhat limit the actual amount of mashed potatoes (say below 350-400 g) to prevent the occurrence of nausea and vomiting.

## 5. Appendices

### Appendix 1. Written Informed Consent Form (Dutch)

Afdeling: Nationaal Vergiftigingen Informatie Centrum (NVIC)

Hoofd: Dr. J. Meulenbelt

#### **INFORMED CONSENT (Bewuste bereidverklaring)**

**Titel onderzoek: "Klinisch pilot onderzoek naar de biobeschikbaarheid van glycoalkaloiden uit aardappelen"; RIVM projectnummer 388802; Datum actuele onderzoeksprotocol: 11 juni 1998**

Ondergetekende verklaart dat hij/zij:

- \* Een **informatiebrief voor vrijwilligers** (gedateerd op **11 juni 1998**) heeft ontvangen over het klinisch pilot onderzoek naar de biobeschikbaarheid van glycoalkaloiden uit aardappelen.
- \* Een **mondelinge toelichting** op de schriftelijke informatie heeft gekregen en voldoende gelegenheid heeft gekregen tot het stellen van vragen.
- \* **Bereid** is om deel te nemen aan het onderzoek en zich te houden aan de opzet en voorschriften van het onderzoek gedurende de gehele onderzoeksperiode, zoals beschreven in de informatiebrief voor vrijwilligers.
- \* **Inzagerecht** verleend in de vertrouwelijke gegevens, aan: de verantwoordelijke artsonderzoeker en andere onderzoeksmedewerkers van het NVIC, en personen of instanties voor kwaliteitsbewaking en -controle (medisch ethische commissie, monitor, auditor).
- \* Bij vragen en/of problemen contact zal opnemen met het Nationaal Vergiftigingen Informatie Centrum (gehuisvest in het Academisch Ziekenhuis Utrecht), tel. 030-2507340 of 2508561 (tijdens kantooruren) en tel. 030-2748888 (buiten kantooruren).
- \* Zich bewust te zijn dat deelname aan het onderzoek vrijwillig is, en dat op elk moment deelname aan het onderzoek kan worden beëindigd (zonder opgave van reden).

(Invullen in BLOKLETTERS)

Naam : .....

Roepnaam : .....

Voornamen (voluit) : .....

Geboortedatum **1 9**

□□□□□□ □□□□□□ □□□□□□□□□□

Dag Maand Jaar

Adres ..... □□□□□□□□□□

Postcode □□□□□□□□□□ □□□□□□

Woonplaats: .....

Utrecht, **1 9**

□□□□□□ □□□□□□ □□□□□□□□□□

Dag Maand Jaar

Handtekening vrijwilliger: Naam en handtekening onderzoeker:

.....

.....



## Appendix 2. Selection criteria for enrolment in the study

### Inclusion criteria:

- Age 18 - 45 years, male or female
- Provision of written informed consent
- Good health according to the clinical investigator
- Willingness and ability to adhere to the study requirements

### Exclusion criteria:

- Pregnancy or lactation (urine pregnancy test for female subjects will be performed just prior to each treatment)
- Deviation from the dietary rules (details described below)
- Evidence of a severe/chronic disease
- Disease and/or surgery of the gastrointestinal tract, liver, or kidneys (with the exception of appendectomy)
- Use of medication (with the exception of oral contraceptives): chronic use, use within 2 weeks prior to the start of the study, or during the study (without agreement of the principle clinical investigator)
- Use of antibiotics within 4 weeks prior to the study and during the study
- Use of narcotics and/or evidence of excessive alcohol abuse
- Asthmatic patients
- Body weight lower than 60 or higher than 80 kg
- Body Mass-index (weight {in kg}/square height {in m})  $\leq 20$  and  $\geq 26$
- Blood donation and/or participation in another study, within 4 weeks prior to the start of the study and during the study
- Poor venous accessibility
- Known sensibility to glycoalkaloids or other potato constituent
- Employee of UMC Utrecht, RIVM, VWS

### Temporary exclusion criterion

Occurrence of an intercurrent disease shortly before (1 week) or during the study, especially in case of an infectious disease and/or complaints/symptoms of the gastrointestinal tract. Volunteers were advised to refrain from blood donation after the study for a period of three months.

## Appendix 3. Preparation procedures of mashed potatoes

### *Composition of potatoes*

Potatoes of the variety 'Elkana' have been used for production of potato flakes.

Potato characteristics of these potatoes have been determined by "Nederlands Instituut voor Koolhydraat Onderzoek" TNO, Groningen:

Dry matter	25.8% m/m
Starch	18.7% m/m
Total sugar	1.51% m/m
Total raw protein	2.83% m/m
Glycoalkaloids:	310 mg/kg <i>unpeeled potatoes</i> (180 mg $\alpha$ -solanine, 110 mg $\alpha$ -chaconine and 20 mg $\beta$ -chaconine)

Potatoes have been steam peeled, boiled, mashed and dried resulting in potato flakes by Agrotechnical Research Institute (ATO-DLO), Wageningen. The potato flakes have been canned under nitrogen atmosphere until use.

### *Recipe of mashed potatoes*

Add 100 g potato flakes to 480 ml hot water (90°C).

Stir until the potato flakes have been hydrated.

Add some salt, pepper [peper] and red pepper [paprikapoeder].

### *Composition of mashed potatoes (per kg)*

828	ml	tap water
171	g	potato flakes containing GAs*
1.1	g	Dimodan**
0.08	g	sulphite (Na <sub>2</sub> S <sub>2</sub> O <sub>5</sub> )
some salt, pepper and red pepper		

\* GAs: approximately 104 mg  $\alpha$ -solanine and approximately 95 mg  $\alpha$ -chaconine per kg *mashed potatoes*.

\*\* Dimodan is an emulsifier which has been added during the production of the potato flakes. The quantity of Dimodan present in mashed potatoes is within the normal usage range for potato products: 0.9% Dimodan of starch (1 kg mashed potatoes contains 124 g starch).

\*\*\* Sulphite (54.0 mg SO<sub>2</sub> equivalents/kg mashed potatoes) has been added during the production of the potato flakes to improve the keeping qualities of the potato flakes.

The acceptable daily intake (ADI) of sulphite is 0-0.7 mg/kg body weight for SO<sub>2</sub> en SO<sub>2</sub> equivalents [44]. In subjects receiving 0.8 mg GAs/kg mashed potatoes the intake of sulphite is within the acceptable range: 0.2 mg/kg body weight for SO<sub>2</sub> and SO<sub>2</sub> equivalents.

## Appendix 4. Protocol Amendment

### PROTOCOL AMENDMENT APPROVAL FORM

March 15th, 1999

Protocol Title: Clinical pilot study on the bioavailability of glycoalkaloids from potato; Date of Issue: June 11th, 1998  
 Protocol Number WOM: 98/133  
 Date of Ethical Board Approval: September 4th, 1998

Protocol Amendment Number: 1  
 Effective Protocol Amendment Date: Date of Ethical Board Amendment Approval

Reason for Amendment: Alteration of the study design, consisting of:

- Adjustment doses glycoalkaloid solution in Dose Step 3 from 0.4 to 0.5 mg glycoalkaloids/kg bodyweight
- Adjustment doses glycoalkaloid solution in Dose Step 4 from 0.5 to 0.7 mg glycoalkaloids/kg bodyweight

**This amendment and its consequences are elucidated on Page 2 of this form.**

This protocol amendment is approved by the following:

Name:	Signature:	Date:
Meulenbelt J, MD, PhD National Poisons Control Centre (RIVM*)	.....	.....
Mensinga TjT, MD, PhD National Poisons Control Centre (RIVM*)	.....	.....
Sips AJAM*, PhD Laboratory of Exposure Assessment (RIVM*)	.....	.....

\* RIVM: National Institute of Public Health and the Environment

† AZU: University Hospital Utrecht

Amendment continued on the next page.

*Amendment continued.*

### Proposed amendment and its consequences

The dose step scheme as described in the original protocol of the presently performed **pilot dose-finding study** (as approved by the medical ethical committee WOM on September 4th, 1998), was as follows (Table 1):

*Table 1, Dose step scheme*

Dose	Orally administered test material	GA-content* (mg/kg bw)
A <sub>1</sub>	Mashed potatoes containing $\alpha$ -solanine and $\alpha$ -chaconine <sup>†</sup>	0.80
B <sub>1</sub>	Solution $\alpha$ -solanine and $\alpha$ - chaconine <sup>†</sup>	0.20
A <sub>2</sub>	Mashed potatoes containing $\alpha$ -solanine and $\alpha$ -chaconine <sup>†</sup>	0.95
B <sub>2</sub>	Solution $\alpha$ -solanine and $\alpha$ - chaconine <sup>†</sup>	0.30
A <sub>3</sub>	Mashed potatoes containing $\alpha$ -solanine and $\alpha$ -chaconine <sup>†</sup>	1.10
B <sub>3</sub>	Solution $\alpha$ -solanine and $\alpha$ - chaconine <sup>†</sup>	0.40
A <sub>4</sub>	Mashed potatoes containing $\alpha$ -solanine and $\alpha$ -chaconine <sup>†</sup>	1.25
B <sub>4</sub>	Solution $\alpha$ -solanine and $\alpha$ - chaconine <sup>†</sup>	0.50

\* GA content =  $\alpha$ -solanine +  $\alpha$ -chaconine: 199 mg/kg potatoes.

<sup>†</sup> Ratio of  $\alpha$ -solanine/ $\alpha$ -chaconine: 52%/48%.

At present Dose Steps A<sub>1</sub>/B<sub>1</sub> and A<sub>2</sub>/B<sub>2</sub> have been performed from respectively February 15-20, 1999 an March 01-06, 1999. In these Dose Steps *no adverse effects* were observed, neither systemic nor local gastrointestinal effects (nausea, vomiting, diarrhoea). The observed concentration-time curves for the administration of mashed potatoes (A<sub>1</sub> and A<sub>2</sub>) almost meets the study goal to adequately describe the biokinetic profile. The performance of Dose Step A<sub>3</sub> will very likely meet the study goal (Dose Step A<sub>4</sub> will probably not be necessary).

For safety reasons, the administration of the solution of glycoalkaloids started with a conservative doses and with conservative dose steps. However, the present intermediate results (of administration of the solution of glycoalkaloids B<sub>1</sub> and B<sub>2</sub>) showed us that the original dose step scheme for the solution of glycoalkaloids was a little too conservative (see Table 2).

*Table 2, Results Dose Step A<sub>2</sub>/B<sub>2</sub>*

Administration Form	Solanine		Chaconine	
Mashed potatoes	C <sub>max</sub> (ng/ml)	9.1	C <sub>max</sub> (ng/ml)	6.5
	AUC (ng*h/ml)	176	AUC (ng*h/ml)	260
Solution	C <sub>max</sub> (ng/ml)	3.4	C <sub>max</sub> (ng/ml)	3.0
	AUC (ng*h/ml)	79	AUC (ng*h/ml)	109

Because we do not expect *adverse effects* to occur we would like to adjust the doses glycoalkaloid solution to meet the study goal of adequate biokinetic profile description, in:

- Dose Step 3 from 0.4 to 0.5 mg glycoalkaloids/kg bodyweight
- Dose Step 4 from 0.5 to 0.7 mg glycoalkaloids/kg bodyweight

The next Dose Step for the solution administration (B<sub>3</sub>) is scheduled on March 16-20, 1999.

## Appendix 5. Dietary rules

Every day, volunteers were asked to complete a short diet questionnaire concerning consumption products containing glycoalkaloids. Further more volunteers are instructed to refrain from eating or drinking (with the exception of water) after 23.00 hours the evening before admission. With the exception of the administered study compounds, the use of the following food products are prohibited 72 hour before admission and during the entire study:

- Potatoes, in any form (boiled, baked, fried, etc.)
- Crisps, and other salty products prepared out of potatoes: French fries, {Wokkels}<sup>\*</sup>
- Soup: ready-made soup [soep uit blik/pak/zak]<sup>\*</sup>
- Salads: potato salad [aardappelsalade], Russian salad [huzarensalade], chicken-curry salad [kip-kerriesalade], salmon salad [zalsalade], etc.
- Other food products containing potato flour [aardappelmeel]

<sup>\*</sup> Dutch product names are presented between [square brackets], while product brand names are presented between {brackets}

The following products were allowed to be used instead of potato: bread, rice pasta, e.g. spaghetti, macaroni, lasagne, etc. or couscous.

## Appendix 6. Demographic data

UTN*	Enrolled in the study	Gender (M/F)	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m <sup>2</sup> )
01	NO	M	23	170	59	20.4
02	NO	F	22	172	78	26.4
03	YES	M	25	187	67	19.2
04	YES	M	24	170	66	22.8
05	YES	F	28	179	71	22.2
06	YES	M	27	185	82	24.0
07	NO	F	22	173	62	20.7
08	NO	M	37	180	80	24.7
09	YES	M	28	173	70	23.4
10	YES	M	21	180	70	21.6
11	YES	F	23	176	79	25.5
12	YES	M	28	180	72	22.2
13	NO	F	45	170	75	26.0
14	YES	M	28	182	72	21.7
15	YES	M	20	184	72	21.3
16	NO	M	21	185	75	21.4
17	YES	F	25	185	79	23.1
18	YES	F	23	174	66	21.8
19	YES	F	27	174	61	20.1
20	YES	F	34	180	71	21.9
21	YES	F	25	181	76	23.2
22	YES	M	23	185	82	24.0
23	YES	F	23	175	62	20.2
24	YES	F	21	181	65	19.8
25	YES	F	20	173	60	20.0
Enrolled subjects			Mean			
Men		N=9	21.8	180.7	72.6	22.2
Women		N=10	24.9	177.8	76.7	21.8

\* Unique trial number

## Appendix 7. Findings at the check-in examination

Clinically relevant findings are listed in the overview below, while details of vital signs and laboratory analyses (haematology, biochemistry, urine) are listed on page 73.

### Appendix 7, Overview clinically relevant findings at the examination of check-in

UTN	Remarks
01	<b>Excluded</b> from the study because of a low cholinesterase level 5.30 and 4.94 kU/L (normal range 5.90-12.20 kU/L) upon repeat 7 days later.
02	<b>Volunteer withdrew from the study before dosing</b> ; reason unknown. CK moderately elevated (432 U/l)
03	Medical history included a hernia inguinalis operation at age 8, a fungal skin infection on the back that was being treated with miconazol cream, a common cold in the week prior to screening and $\gamma$ -globulin immunisation therapy a month prior to dosing. Subject included.
04	Medical history included hay fever and an allergy to grapes. Moderately elevated CK level 452 U/L likely due to sport activities. Subject included.
05	Medical history included a mild throat inflammation in the week prior to the screening with some residual complaints on the day of the screening. Physical examination showed a clear throat. Moderately elevated ESR 21 mm/hr unchanged upon repeat 7 days later. Subject included.
06	Physical examination showed a bodyweight of 82 kg which was 2 kg too heavy, but a BMI of 24.0 (20-27 kg/m <sup>2</sup> ). The ECG recorded non-specific conduction disturbance in the inferior wall, regarded to be not clinically significant. Subject included
07	<b>Volunteer withdrew from the study before dosing</b> ; reason unknown. Medical history included a cold in the week prior to screening. Physical examination of the lungs showed a mild expiratory wheeze. Laboratory results showed a low lymphocyte count of 14% accompanied by a slightly high neutrophile count of 76% (possibly mild bronchitis).
08	<b>Volunteer withdrew from the study before dosing</b> ; reason unknown.
09	Medical history included allergy to cats and dogs that was being treated with monthly hyposensitisation injection. Latest treatment 14 days before dosing. Subject included.
10	Medical history included hay-fever, occasional eczema, a productive smokers cough and a common cold with mild fever in the week prior to screening. Subject included.
11	Medical history included surgery of the jaw at age 19. Physical examination showed scars due to bilateral mammoplasty and slightly enlarged submandibular lymph nodes. The ECG recorded a short PR-interval with a normal sinus rhythm. Laboratory results showed low K 3.1 and 3.6 mmol/L (3.8-5.0 mmol/L) upon repeat 6 days later. Subject included.
12	Physical examination showed some slightly enlarged lymph nodes in the neck but was otherwise unremarkable. The ECG recorded a right atrial hypertrophy. Laboratory results showed a slightly raised monocyte count of 15%. Subject included.
13	<b>Volunteer withdrawal before dosing due to illness</b> (unspecified). Medical history included a haemoroïdectomy, hysterectomy and arthroscopy of the knee. Physical examination showed a slight homogeneously enlarged thyroid.
14	Laboratory results showed cholinesterase of 5.60 kU/L, and 6.21 kU/L upon repeat 16 days later (normal range 5.9-12.20 kU/L). Subject included.
15	Medical screening unremarkable. Subject included.
16	<b>Excluded</b> from the study. The laboratory results showed a low cholinesterase level 5.3 kU/L (5.90-12.20 kU/L). The ECG recorded an irregular sinus rhythm, with a moderate conduction disturbance at the level of the His-bundle and occasional ectopic beats. Volunteer was referred to his GP for further assessment.

Appendix 7, *Continued*, Overview clinical relevant findings at the examination of check-in

UTN	Remarks
17	Medical history included a clavicle fracture at age 20, excision of a benign naevus on the right arm at age 24 and a mild common cold in the week prior to the screening. Physical examination showed the scar on the right arm but was otherwise unremarkable. Laboratory results showed a slightly low K 3.4 mmol/L (3.8-5.0 mmol/L). Subject included.
18	Medical history only included hay fever but was otherwise unremarkable. Subject included.
19	Laboratory results showed a slightly low albumin 33.4 g/L (35.0-50.0 g/L), a slightly raised ESR 16 mm/hr in a female subject, and a slightly elevated lymphocyte count of 49%. Subject included.
20	Medical history included tonsillectomy at age 30 and episcleritis of the left-eye that was being treated with dexamethasone eye drops. Subject included.
21	Medical history included a mild contusio cerebri at age 17, occasional complaints of an irregular heart rate and a common cold with a throat inflammation in the week prior to screening. Physical examination and the ECG were unremarkable. The K level was 3.2 and 3.5 (3.8-5.0 mmol/L) upon repeat 6 days later. Laboratory results furthermore showed a slightly raised neutrophil and monocyte count at the expense of lymphocytes. The urinalysis contained some leukocytes (10-25/ $\mu$ L). Subject included.
22	Medical history included tonsillectomy at age 7. Physical examination showed a bodyweight of 82 kg which was 2 kg too heavy but a BMI of 24.0 (20-27 kg/m <sup>2</sup> ). Subject included.
23	Medical history included a mild flu in the week prior to the screening. Physical examination showed a mild dry cough with clear lungs. Laboratory results showed slightly raised lymphocyte and eosinophile counts of respectively of 57% and 14%; and on repeat on day 1 before the dosing respectively 40% and 15%. Subject included.
24	Medical history included viral meningitis at age 10, urethra dilatation at age 8 and 12, occasional mild migraines that require no medical therapy and a common cold in the week prior to screening. Subject included.
25	Medical history included surgery to correct a protruding sternum at age 12, arthroscopy of the left-knee at age 18 and hay fever. The physical examination showed the scars from the sternum and knee surgery. Subject included.





## Appendix 8. Cholinesterase concentrations

Appendix 8, Cholinesterase concentrations\*

UTN (M/F)	Check-in		During the study at time points			Check-out
	Pre-study	Repeat	-1 hour	+7 hours	+24 hours	Post-study
03 (M)	7.95		6.85	6.85	7.25	7.70
04 (M)	9.26	8.16	7.31	7.50	7.71	7.54
05 (F)	7.08	8.06	7.35	7.80	6.99	6.77
06 (M)	8.79	8.81	8.33	8.19	7.83	7.94
09 (M)	9.34		8.99	9.28	10.24	9.69
10 (M)	7.13	6.77	7.21	6.24	6.46	7.00
11 (F)	6.65	6.80	6.44	5.54	5.42	6.85
12 (M)	9.65		9.10	8.58	9.11	8.58
14 (M)	5.60	6.21	6.43	6.10	6.19	5.36
15 (M)	7.36		6.22	6.59	6.81	NA <sup>†</sup>
17 (F)	7.42		7.22	6.96	7.43	7.40
18 (F)	5.76		6.39	6.62	6.49	5.63
19 (F)	6.67		6.04	6.09	6.48	6.28
20 (F)	6.22		6.19	6.48	6.73	7.13
21 (F)	6.24		7.70	6.41	7.06	7.62
22 (M)	7.04		6.46	6.37	6.93	6.51
23 (F)	4.95		6.46	7.10	6.86	5.85
24 (F)	5.44		5.18	5.50	5.43	4.86
25 (F)	5.45		5.09	5.41	5.32	4.25

\* Normal range: Males (5.9-12.2 kU/L), females (4.7-10.4 kU/L).

† NA= not available

## Appendix 9. Findings at the check-out examination

Clinically relevant findings are listed in the overview below, while details of vital signs and laboratory analyses (haematology, biochemistry, urine) are listed on page 76.

### Appendix 9, Overview clinically relevant findings at the examination of check-out

UTN	Remarks
03	<i>A 3 cm patch of a fungal skin infection on the left thoracic side of the back, to be treated by GP.</i>
04	Slightly elevated CK now at 328. Not clinically relevant.
06	Slightly elevated CK 234 U/l. Not clinically relevant.
11	<i>Slightly elevated ESR 17 mm/h and total protein 84 g/l. Slightly low level of K 3.4 mmol/l and slightly high neutrophil count 75%. Not clinically relevant.</i>
15	<i>Healthy subject on examination, laboratory results not available.</i>
17	<i>Physical examination showed some residual oedema and erythema of the eyelid as well as some urticaria with scratch marks on the left side of the neck, caused by the facial night cream used. Further evaluation by GP.</i>
19	Slightly elevated lymphocyte count 56%, in a furthermore healthy subject. Further evaluation by GP.
23	<b>Palpable submandibular lymph nodes. Slightly elevated eosinophil count 12%. Not clinically relevant.</b>
25	<i>Slightly low cholinesterase level of 4.25 kU/l. Not clinically relevant.</i>

Appendix 9, Continued

Vital signs and laboratory variables at Check-in examination

UTN	Vital signs				Haematology										Biochemistry													
	HR	BS	BD		Hb	Ht	Th	Le	Eo	Ba	Ne	Ly	Mo	ESR	Gl	Ur	Cr	Na	K	Cl	Bi	AP	GT	AS	AL	Pr	Alb	CK
3	82	121	66		9.7	0.44	229	7.2	3	1	59	24	13	3	4.8	5.5	70	140	3.9	101	29.7	58	20	25	20	80	49.0	97
4	66	110	55		9.0	0.41	214	4.5	2	1	59	31	9	2	4.9	5.0	82	144	3.8	107	25.3	70	18	39	22	74	40.7	328
5	87	112	63		7.8	0.36	282	---	0	2	50	41	7	9	4.5	3.4	69	142	4.1	108	26.4	64	26	22	20	76	38.3	150
6	75	132	71		8.8	0.40	261	8.5	2	0	67	24	7	3	4.9	7.3	113	142	4.0	103	26.9	64	8	34	28	72	40.9	234
9	81	117	62		8.4	0.45	258	5.9	3	0	53	34	9	4	4.8	4.5	87	142	4.0	102	29.2	55	21	21	22	75	40.9	99
10	59	129	64		9.7	0.45	238	6.4	2	1	54	33	10	3	5.0	4.7	81	137	3.8	99	30.4	69	19	21	19	79	45.7	148
11	76	139	80		9.1	0.42	281	9.8	1	1	75	17	6	17	4.5	3.9	69	138	3.4	99	29.3	94	25	22	22	84	43.2	51
12	81	132	64		9.4	0.42	185	3.7	5	0	48	33	14	5	4.4	4.4	83	141	4.0	104	30.7	58	19	22	23	75	41.1	53
14	77	118	71		8.9	0.42	150	4.4	2	0	51	33	14	6	5.3	5.0	85	142	3.6	104	27.6	60	16	25	16	71	39.0	103
15	71	116	67		---	---	---	---	-	-	-	-	-	-	---	---	---	---	---	---	---	---	---	---	---	---	---	---
17	70	118	70		9.5	0.44	246	5.5	4	1	46	40	9	6	4.2	3.3	71	141	3.6	106	29.4	61	14	16	17	74	38.1	84
18	75	127	66		7.7	0.36	309	7.0	2	1	61	32	4	4	4.8	5.6	65	138	4.0	104	28.0	62	11	24	23	76	41.3	191
19	88	119	71		8.6	0.30	261	6.6	3	1	36	56	5	6	3.6	4.0	75	140	3.8	105	30.5	53	16	21	15	72	34.1	100
20	78	127	64		8.3	0.38	197	6.7	2	0	50	34	14	5	5.1	3.9	80	139	3.8	102	29.7	56	17	27	21	77	44.4	152
21	69	123	71		8.1	0.37	332	5.0	3	2	48	35	12	12	4.3	4.0	62	140	3.8	102	30.3	56	16	23	30	74	39.7	67
22	66	117	57		9.2	0.42	205	5.2	3	1	51	36	9	3	2.9	4.7	78	140	3.6	100	32.2	71	22	41	31	76	41.8	380
23	76	121	68		7.9	0.37	258	---	12	2	37	44	5	6	3.7	4.8	81	139	3.7	105	28.4	44	14	20	17	72	35.9	133
24	57	102	61		7.5	0.36	272	7.4	14	1	44	36	5	6	3.8	2.6	67	139	4.4	105	28.6	50	28	24	31	75	37.6	40
25	73	107	55		7.4	0.35	274	6.9	2	1	59	29	9	7	4.6	5.2	60	140	4.1	108	27.1	52	11	25	15	68	33.8	60
clinically relevant					Haematology [normal values]										Biochemistry [normal values]:													
aberrant test results					Hb:	Haemoglobin [M 8.6-10.7, F 7.4-9.6] mmol/l									Gl = Glucose [3.6-5.6] mmol/l (fasting)													
<u>underlined</u>					Ht:	Haematocrit [M 0.41-0.55, F 0.36-0.46] l/l									Ur = Urea [3.0-7.5] mmol/l													
					Th:	Thrombocyte count [150-450] *10 <sup>9</sup> /l									Cr = Creatinine [50-120] mmol/l													
					Le:	Leukocyte count [4.0-10.0] *10 <sup>9</sup> /l									Na = Sodium [136-146] mmol/l													
					Eo:	Eosinophils [0-5] %									K = Potassium [3.8-5.0] mmol/l													
					Ba:	Basophils [0-2] %									Cl = Chloride [99-108] mmol/l													
					Ne:	Neutrophils [40-72] %									Bi = Bicarbonate [23-29] mmol/l													
					Ly:	Lymphocytes [20-45] %									AP = Alkaline Phosphatase [40-130] U/l													
					Mo:	Monocytes [3-10] %									GT = Gamma GT [M 15-70, F 15-45] U/l													
					ESR:	Erythrocyte Sedimentation Rate [M 1-5, F 2-12]									AS = ASAT [15-45] U/l													
															AL = ALAT [10-50] U/l													
															Pr = Protein [62-78] g/l													
					BD:	Diastolic Blood pressure									Alb= Albumin [35-50 g/l]													
															CK = Creatinine Phosphokinase [15-180] U/l													

## References

1. Dalvi RR, Bowie WC. Toxicology of solanine: An overview. *Veterinary and Human Toxicology* 1983;25:13-15.
2. Van Gelder WMJ. Steroidal Glycoalkaloids in Solanum. In: Keeler RF TA, editor. *Toxicology of plant and fungal compounds*. New York: Marcel Dekker Inc; 1991. p. 101-35.
3. Valkonen JPT KMVTPL. Potato Glycoalkaloids: A Burden or a Blessing? *Critical Reviews in Plant Sciences* 1996;15(1):1-20.
4. Jadhav SJ SRSD. Naturally occurring toxic alkaloids in foods. *CRC Critical Reviews in Toxicology* 1981;9:21-104.
5. Johnson H HK. Glycoalkaloids in potato. *Var Föda* 1983;35:299-314.
6. Zitnak A. The occurrence and distribution of free alkaloid solanidine in Netted Gem potatoes. *Can J Biochem Physiol* 1961;39:1257-65.
7. Wood FA YD. TGA in potatoes. *Can Dep Agric Publ* 1974: 1533.
8. Friedman M MDG. Potato glycoalkaloids: chemistry, analysis, safety and plant physiology. *Critical Reviews in Plant Sciences* 1997;16:55-132.
9. Wünsch A, Munzert M. Einfluss von Hagerung und Sorte auf die Verteilung der Glycoalkaloide in der Kartoffelknolle. *Potato Res* 1994;37, 3-10.
10. Sinden SL DKAB. Effect of glycoalkaloids and phenolics on potato flavor. *J Food Sci* 1976;41:520-3.
11. Sizer CE MJCC. Total glycoalkaloids in potatoes and potato chips. *J Agric Food Chem* 1980;28:578-9.
12. Bushway RJ, Barden ES, Bushway AW, Bushway AA. The mass extraction of potato glycoalkaloids from blossoms. *Am.Potato J.* 1980;57 :175-80.
13. Zitnak A, Johnston GR. Glycoalkaloid content of B5141-6 potatoes. *Am.Potato J.* 1970;47: 256-60.
14. Nishie K, GumbmannMR Keyl AC. Pharmacology of solanine. *Toxic.appl.Pharmac* 1971;19, 81-92.
15. Nishie K NWSA. Pharmacology and Toxicology of Chaconine and Tomatine. *Research Communications in Chem Path.and Pharm* 1975;12(4):657-68.
16. Bömer A, Mattis H. The solanine content of potatoes. *Z.Unters.Nahr-.u.Genussmittel* 1924;47:97-127.
17. CBS. *Statistisch Jaarboek*. Voorburg/Heerlen: Centraal Bureau voor de Statistiek; 1997.
18. McMillan M TJ. An outbreak of suspected solanine poisoning in schoolboys: examination of criteria of solanine poisoning. *Quarterly Journal of Medicine* 1979;48:227-43.
19. Morris SC and Lee TH. The toxicity and teratogenicity of Solanaceae glycoalkaloids particularly those of the potato (*Solanum tuberosum*): a review. *Food Technol Aust* 1984;36:118-24.
20. WHO. JECFA: Toxicological evaluation of certain food additives and naturally occurring toxicants. *WHO Food Additives Series* 1993;30:339-72.
21. Hellenäs K-E NASPLLJGJ. Determination of potato glycoalkaloids and their aglycone in blood serum by high-performance liquid chromatography. Application to pharmacokinetic studies in humans. *Journal of Chromatography* 1992;573:69-78.