COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

WORKING GROUP ON PHYTOESTROGENS

ABSORPTION, DISTRIBUTION, METABOLISM & EXCRETION OF PHYTOESTROGENS

Introduction

1. A review of the absorption, distribution, metabolism and excretion (ADME) of phytoestrogens has been prepared by the Department of Biochemical Pharmacology, Imperial College School of Medicine and is attached at Annex 1. The paper also includes a review of the role of gut microflora and inter-individual differences in these processes.

2. The 1996 COT review highlighted the need for further research into the ADME of phytoestrogens, and in 1997, MAFF funded the following projects to address the COT recommendations. This research is now the responsibility of the Food Standards Agency.

   ADME of \([^{14}\text{C}]-\text{genistein}\) in male and female rats. (Veterinary Laboratories Agency)

Two papers on the metabolism of genistein in the rat have been published from this research. The papers are attached as Annex 2 & 3.

   Absorption, distribution, metabolism and excretion of isoflavones in vivo. (University of Surrey): Identification and measurement of daidzein, genistein and metabolites in human blood, urine and faeces following acute and chronic exposure.

This research is still underway but the final results should be available for consideration at the November meeting.

3. Absorption & Metabolism

In general, phytoestrogens are ingested as conjugates and are thought to require hydrolysis prior to absorption. Hydrolysis is thought to occur in the stomach & lower gut. Microflora-mediated reduction, demethylation and dehydroxylation reactions occur prior to absorption.
Absorbed phytoestrogens are then reconjugated in the liver and intestinal epithelium by glucuronosyl and sulpho transferases. Plasma concentrations of free phytoestrogens are low.

**Isoflavones**
Genistein and daidzein undergo reductive biotransformations. Genistein is converted to 4-ethylphenol via dihydrogenistein and 6'-hydroxy-O-demethylangolensin. Daidzein is converted to 4-ethylphenol via similar pathways. Daidzein is converted to equol in approximately 30% of humans.

**Lignans**
Lignans per se have not been shown to be estrogenically active. Microflora mediated dehydroxylation and demethylation produce the “active” compounds, enterolactone and enterodiol. Glucuronides of these compounds have been identified.

**Coumestans**
Little is known about the metabolism of coumestans.

**Prenylisoflavonoids**
Little is known about the metabolism of prenylisoflavonoids.

4. **Excretion & Enterohepatic Circulation**

Phytoestrogen conjugates and reduction products are excreted in urine and faeces. Excretion of phytoestrogens follows food intake. Conjugates of lignans and their metabolites have also been identified in human urine. In Western populations, urinary levels of lignans are greater than isoflavones. The converse is true for Japanese populations.

Biliary conjugates can undergo deconjugation in the gut & metabolised further or reabsorbed, prolonging exposure. The extent of enterohepatic recirculation determines the proportion of faecal elimination.

5. **Distribution**

Very little information is available on the tissue distribution of phytoestrogens. Foetal exposure to phytoestrogens has been reported. Lignans, genistein, daidzein and metabolites have been detected in neonatal umbilical cord, amniotic fluid in levels similar to maternal plasma.

6. **Pharmacokinetics**

Few comprehensive pharmacokinetic studies have been undertaken. The most comprehensive study to date is that by Watanabe et al in 1998, but other published studies generally support these findings. The Food Standards Agency has commissioned a study into the ADME of isoflavones in humans.
which should be available for discussion at the November meeting of the Working Group.

7. **Summary of the Watanabe data on isoflavones**

Adult males (n=7) were given a single oral dose of baked soybean powder and levels of genistein, daidzein, O-desmethylangolensin and equol were measured in plasma, urine and faeces. The recoveries of genistein and daidzein plus metabolites were estimated at ~20% and ~55%, respectively. Indicating that not all metabolites had been identified.

8. **Plasma**

Increased plasma levels of isoflavones were seen within 2 hours of dosing. Maximal plasma concentrations of genistein and daidzein were reached in ~8 hours. Peaks in metabolite concentration followed 1-2 hours thereafter. Half-lives of genistein and daidzein were 8.4 and 5.8 hours, respectively. Phytoestrogen concentrations approached basal levels after 24 hours. Inter-individual variations in equol and O-desmethylangolensin production ranged from ~0-66% and ~7-21%, respectively. Plasma concentration patterns indicated enterohepatic circulation.

9. **Excretion**

Urinary excretion accounted for the majority of the identified excreted compounds (36% and 18% of ingested daidzein and genistein, respectively). Urinary excretion varied considerably and peaked 6-12 hours after ingestion. Basal levels were reached after 24 hours. Excretion patterns indicated enterohepatic circulation. Most of the isoflavone-derived excretion in faeces occurred on the second and third days following ingestion (4.5% and 2.5% of ingested daidzein and genistein, respectively).

10. **Lignans**

Little information is available on lignan pharmacokinetics. Peak plasma concentrations of enterolactone and enterodiol occur at ~8-9 hours post-ingestion. Urinary excretion follows a dose dependent pattern and is maximal within 24 hours of ingestion. No studies have been carried out to investigate if lignans can transfer into breast milk.

11. **Bioavailability**

The bioavailability of phytoestrogens may be influenced by food matrix and phytoestrogen conjugation patterns, however the current data is conflicting. Two Food Standards Agency funded research projects are currently investigating this particular topic.

12. **Transfer to Breast Milk**

This paper has been prepared for discussion by the COT Working Group on Phytoestrogens and does not necessarily reflect the final views of the Group.
Genistein, daidzein and metabolites have been detected in breast milk and appear to transfer in a dose dependent manner. Metabolites in breast milk are similar to those detected in plasma. Maximum levels are detected at 10-14 hours after ingestion. Basal levels are reached after 2-4 days.

13. Inter-individual Variation in Metabolism of Phytoestrogens

Gut microflora greatly influence the ADME of phytoestrogens & there is considerable inter-individual variation in gut microflora composition. Gut-flora are responsible for the production of equol and the O-desmethylangolensin metabolites, hence metabolic profiles are dependent on the type of microflora activities present in the gut. Equol producing microflora are present in ~ 30% of the population. Little is known about the role of gut microflora mediated metabolism in the conversion of dietary lignans to active metabolites. However, it is clear that differences in gut microflora are responsible for differential enterodiol and enterolactone production.

Development of Gut Microflora
Microflora develop within a week of birth and throughout infancy to adulthood. Colonisation is dependent numerous factors such as maternal gut flora, hygiene, environment, genetics and food. Microflora are more diverse in formula fed infants. Subsequent dietary influences are greater on the gut flora of breast-fed infants.

Factors Affecting Gut Microflora
Diet, hygiene, anti-biotic use, disease, stress, gastric pH, mucin and bile secretion, sex and genetics influence the type and number of bacteria in the gut.

Diet
Production of equol correlates with a low-fat, high carbohydrate (as proportion of total energy intake) diet. Low-fat diets may lower glucuronidase activity and reduce absorption of hydrophobic compounds. Dietary fibre appears to reduce absorption and resorption of phytoestrogens by disrupting enterohepatic circulation.

Sex & age differences in metabolism
Sex and age differences in phytoestrogen excretion patterns have been reported however, the data derives from small or single studies.

14. Review of ADME Study of $[^{14}\text{C}]$-Genistein in Rats

The Food Standards Agency has funded a study into the ADME of $[^{14}\text{C}]$-genistein in male and female rats. This was carried out by researchers at the Veterinary Laboratories Agency and papers describing the identity of the metabolites (annex 2) and the pharmacokinetics (annex 3) are appended.
A single dose of 4mg/kg bw $[^{14}C]$-genistein (equivalent to calculated intake of genistein by human infants on soya-based (infant formula) dose was administered by gavage to male and female rats (each n= 5).

**Mass Balance**
Mean % of the dose were in found in in the urine, faeces and carcass was, 67, 31 and <1 % in males and 66, 36 and <1 % in females after 168 hours.

**Plasma Pharmacokinetics**
$[^{14}C]$-Genistein was quickly (0.5-2.5 hours) absorbed and distributed in the plasma. A further peak in plasma concentration was observed at 6 – 8 hours. Plasma half-life of $[^{14}C]$-genistein was 12.4 hours for males and 8.5 hours for females and approached basal levels after 24 hours.

**Tissue Distribution**
The radioactive content of GI tract, excretory, respiratory, peripheral and reproductive organs at 2, 7 and 24 hours is described.

**Plasma Protein Binding**
Radioactivity was predominantly associated with plasma proteins and appeared to be higher in males.

**Biotransformation**
LC-MS was used to identify the metabolites. Genistein was sequentially converted to dihydrogenistein, hydroxy-O-desmethylangolensin and hydroxyphenyl propionic acid by gut microflora. Genistein was also converted to glucuronide and sulphate conjugates and dihydrogenistein to a glucuronide in the liver and intestine. Significant differences in male and female metabolism were noted.

Members are invited to comment on PEG/2000/09. Suggested topics for discussion may include:

Correlation of plasma and urine levels.

Phytoestrogen transfer to the foetus & implications.

Phytoestrogen bioavailability.

Assessment of human exposure given the inter-individual variations in gut micro-flora.

Applicability of the $[^{14}C]$-genistein rodent studies to humans.

Members are also invited to suggest areas that require further research.
Ongoing & Future Food Standards Agency Funded Phytoestrogen Research

Members may wish to be aware that a number of projects in the Food Standards Agency Phytoestrogen R & D programme are currently underway into the ADME of phytoestrogens. The final results will not be available until at least 2001/2002

Absorption and metabolism of dietary phytoestrogens in humans- effect of age, gender, food matrix and chemical composition. (University of Surrey)

Influence of human gut microflora on dietary soya isoflavone phytoestrogen bioavailability in adults and children. (King’s College London)

Investigations on the effects of dose, food matrix, age and gender on isoflavone levels in human blood, urine and faeces.

Identification and quantitation of dietary lignans by LC-MS. (Veterinary Laboratories Agency)

Preliminary investigations on the form, activity and biotransformations of dietary lignans.

Secretariat

June 2000