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

COT STATEMENT ON THE FOOD STANDARDS AGENCY PHYTOESTROGENS RESEARCH PROGRAMME

1. Phytoestrogens are naturally occurring compounds found in some plant-based foods, notably soya. These compounds, as their name suggests, have structural similarities to the female sex hormone, oestradiol. This has prompted concern that consuming phytoestrogens might have oestrogenic, anti-oestrogenic and/or other effects in humans. These effects could be either adverse or beneficial and might differ in particular subgroups of the population.

2. The Phytoestrogen Research Programme (T05/T06) was established to improve the assessment of the risks and benefits from dietary phytoestrogens and the scientific evidence base underpinning advice to consumers. It was subsequently decided that evidence for claimed benefits was the responsibility of the food industry or retailers and that future work on risks should be incorporated into the Food Standards Agency (FSA) Risk Assessment Research Programme (T01). The majority of the research was reviewed in 2001-7. In 2011 the Committee was asked to review briefly the final projects, and to consider the overall contribution of the Phytoestrogen Research Programme to risk assessment for phytoestrogens.

Aims and timeline of the Phytoestrogen Research Programme

3. The Phytoestrogen Research Programme was established in 1997 by the Ministry of Agriculture, Fisheries and Food (MAFF). Its specific remit was to address the following policy objectives:

- The development of suitable analytical methods for phytoestrogens
- The determination of levels of phytoestrogens in food
- Studies on the absorption, distribution, metabolism and excretion (ADME) of phytoestrogens in the body
- Investigation of possible beneficial and/or detrimental effects of phytoestrogens in the general population and in specific (genetic and/or age) subgroups within the population

4. Responsibility for the Phytoestrogen Research Programme transferred to the FSA in 2000. In 2005/6, as the remit of the programme was no longer consistent with the FSA's strategy, it was decided to transfer work on the risks of phytoestrogens to the Risk Assessment Research Programme and that no new work would be commissioned under the Phytoestrogen Research Programme. A list of projects funded in the Phytoestrogen Research Programme and their main findings can be found at Annex1.

5. The Phytoestrogen Research Programme was previously reviewed in 2001 (by externally commissioned contractors), in 2003 (by a COT working group on phytoestrogens and health), and in 2007 together with the Risk Assessment Research Programme (again by an external panel). These earlier reviews are summarised below.

2000-2001 "consensus review" of the phytoestrogens programme

6. In 2000-2001, the FSA commissioned external contractors to review the Phytoestrogens Research Programme (T05018) and carry out a survey of phytoestrogen research being conducted worldwide so that the FSA-funded research could be assessed in the context of global efforts[1]. The reviewers concluded that the Phytoestrogens Research Programme constituted a network of excellence in global research on phytoestrogens. Highlighted as being of particular value were projects focused on the synthesis of phytoestrogen standards and the use of transgenic mouse models containing reporter genes.

7. Dividing the phytoestrogens research into six key areas, the reviewers made the following recommendations for future research in the Phytoestrogens Research Programme:

Analytical and Chemistry

The programme should continue to provide leadership in quality control of existing analytical methods and individual laboratories. Research on the synthesis of pure phytoestrogen standards and those that are multiple Carbon-13 (13C) or Carbon-14 (14C)-labelled should be extended to all phytoestrogens and their metabolites.

Phytoestrogen Intake

Assessment of intake of phytoestrogens cannot be made by out of date Food Frequency Questionnaires (FFQs). Continuing validation of FFQs is essential for all epidemiological studies with large subject groups.

[1] http://www.food.gov.uk/science/research/foodcomponentsresearch/phytoestrogensresearch/t05-t06programme/t05t06projectlist/t05018project/

Absorption, distribution, metabolism and excretion

There is a strong need to use the multiply-labelled forms of genistein and other phytoestrogens to identify their metabolic products in humans. The relationship of equol and other phytoestrogen metabolites to disease risk and disease symptomology is an important issue to pursue. Drug-phytoestrogen interaction in transport processes in the liver and kidney should be investigated in human studies.

Mechanisms

The use of the existing transgenic model containing an estrogen responsive element (ERE) beta-galactosidase reporter gene construct to evaluate estrogen receptor (ER)-mediated effects of phytoestrogens in vivo is an excellent initiative. DNA microarrays and proteomics/mass spectrometry should continue to be used to assess the effects of phytoestrogens in cellular and tissue targets in isolated cells, animal experiments and in clinical trials.

Beneficial effects

Since a growing part of the population are elderly and are consuming soy products for the first time, it is important to carefully monitor the effects of phytoestrogens in this group. Intervention studies with phytoestrogen-containing foods or extracts will provide a larger, more controlled dose range than epidemiological studies where intake is determined by the subjects and may be low. The value of soy in reducing hypertension should be investigated. Further epidemiological studies of bladder, endometrial and thyroid cancers are warranted if concerns about ranges of phytoestrogen intake can be addressed.

Adverse effects

DNA microarrays and proteomics will provide more global information about the adverse effects of phytoestrogens on the biochemistry, biology and pathophysiology in animal models and in clinical investigations. The use of the transgenic mouse model to evaluate ER-mediated effects of phytoestrogens in vivo should yield relevant information. Toxicity testing should be focused on experiments using oral administration since the purpose of this research programme is to determine the risk to the public of foods containing phytoestrogens.

8. Most projects in the Phytoestrogens research programme were ongoing or only recently commissioned at the time of this first review.

2003 COT report on Phytoestrogens

9. During 2000-2003, a COT working group reviewed the available scientific literature, the research funded in the Phytoestrogens Research Programme and the

findings of the 2001 consensus review. A COT report, including recommendations for further research, was published in 2003[2]

10. The COT report recommended research to address important outstanding questions, to aid future risk assessment of dietary phytoestrogens. The report concluded that the majority of published animal studies examining the effects of phytoestrogens could not be extrapolated to humans, and advised that future research should be conducted in humans where possible.

11. In response to these recommendations, the FSA commissioned three further research projects, assessing the relationship between phytoestrogen intake and risk of breast and prostate cancer (T05028), potential effects in individuals with compensated hypothyroidism (T05029), and phytoestrogen exposure in women diagnosed with breast cancer (T05030). Recommendations from the 2001 review were adopted in these new projects, including the synthesis of additional phytoestrogen standards, use of food diaries rather than questionnaires for assessment of phytoestrogen intake, and intervention with phytoestrogen-containing supplements.

2007 Phytoestrogens Research Programme Review

12. The three new projects were ongoing when the Phytoestrogens Research Programme was subject to a further external review in 2007, together with the Risk Assessment Research Programme. The external review panel, which comprised 14 independent experts with relevant experience in toxicology and/or other scientific disciplines, assessed the relative strengths and weaknesses of the Phytoestrogens Research Programme on the basis of the projects that they reviewed.

13. This 2007 review covered the three new projects, together with those which had been at an early stage at the time of the previous review. A report of the review has been published[3]. Extracts from that report, including the general discussion and points made about individual projects, are set out below.

General discussion

Although several of the projects examining biological effects of phytoestrogens in vitro and in vivo had encountered problems, such difficulties had been addressed appropriately. These types of study were considered to be of comparable quality to similar work conducted in research areas other than phytoestrogens.

Although the past and present clinical studies were limited by small sample sizes, that they had value in assessing effects on important endpoints, some of which have not been addressed by other researchers in the UK or internationally.

[2] http://cot.food.gov.uk/cotreports/cotwgreports/phytoestrogensandhealthcot
[3] http://www.food.gov.uk/science/research/foodcomponentsresearch/riskassessment/t01t05t09review/

A significant strength of the programme had been the development of analytical standards for a wide range of phytoestrogens. This has enabled the accurate quantitation of phytoestrogens in foods, supplements and biofluids for the first time, and the standards have been used in Food Standards Agency-funded projects and studies funded by other bodies. Much of this research would not have been possible had these standards not been developed.

The use of transgenic models as a general approach for the assessment of phytoestrogens, xenoestrogens and other xenobiotics was discussed. Such models were seen as a valuable tool that should be included in future FSA-funded projects where appropriate. However, given the technical difficulties in developing such systems, as seen with the T05 projects that had been discussed, it was suggested that the greatest value for money may be achieved by funding projects utilising models that have already been developed and validated, rather than funding model development.

T05014: The effect of phytoestrogen ingestion on menopausal symptoms

Overall, this project was considered to be of good scientific quality, meeting the majority of its objectives and those of the original research requirement. The observed reduction in hot flushes associated with flaxseed supplementation suggests that lignans may have beneficial effects on menopausal symptoms, and it was disappointing that the results had not yet been published in a peer-reviewed journal. While the researchers had discussed the results in terms of statistical significance, it was suggested that it would have been useful to have included greater discussion of their clinical significance. The findings were considered worthy of further investigation, for example in a larger study using a range of lignan doses. However, the panel recognised that research into potential beneficial effects of phytoestrogens or other food chemicals would not fall under the remit of the T01 Risk Assessment programme and would need to be funded by other sources.

T05016: Effects of phytoestrogens on estrogen receptor (ER) mediated gene transcription and protein expression

Although transgenic cell lines and mouse strains had been developed as set out in the Scope of Work[4], a number of technical problems were encountered and several were not responsive to transgene inducers. As a result, the project had not been able to address all of the questions relating to effects of phytoestrogens that were in the original research plan. Nevertheless, the work was considered to be of high quality overall, and where experiments were unsuccessful alternative approaches were taken that shed useful light on the problems encountered. The researchers discussed possible solutions to these problems in the final report and this was seen as adding value for money as it provides a starting point for further research.

[4] The Scope of Work is that part of the research contract that describes the work to be carried out.

T05023: Synthesis of standard phytoestrogens in labelled and unlabelled form

The panel considered this to be excellent work of high scientific and technical quality. The standards developed have supported a number of other studies in the T05 research programme and elsewhere, enabling accurate assessment of both phytoestrogen levels in food and bioavailability and metabolism. In addition to the value of this work for phytoestrogens research, the novel pathways for synthesis of 13C labelled compounds that the researchers have developed and published also represent a significant achievement in the field of organic synthesis. Further work synthesising additional phytoestrogens and their metabolites was supported.

T05024: Quality assurance scheme for the phytoestrogen research programme

The quality assurance scheme was seen as being good value for money, producing useful information on the performance of the analytical laboratories involved in the T05 programme. However, it was suggested that it would be more accurate to describe the scheme as a proficiency scheme rather than quality assurance.

T05025: Effect of ER beta overexpression on molecular action of phytoestrogens

A number of unexpected technical problems had been encountered and as a result this project did not achieve most of its objectives. Nevertheless, it was clear the investigators had taken appropriate steps in attempting to overcome the difficulties encountered. While noting that it may also have been useful to try some alternative approaches to those taken, the panel recognised that it was easy to criticise with hindsight and a logical choice of methods had been used at the time. The overall area of ER alpha and beta gene expression ratios and their association with biological processes and disease was considered worthy of further research.

T05028: Dietary and biomarker prospective study of phytoestrogens in breast and prostate cancer

This project was ongoing at the time of the review and reviewers made only limited comments. Concern was expressed that the amount of time devoted to development of the liquid chromatography – mass spectrometry (LC-MS) method to be used for analysis of phytoestrogens in food seemed somewhat excessive, particularly given that the work was being performed at an experienced laboratory. It was uncertain from the information available to reviewers whether the study would have sufficient power to detect significant differences in phytoestrogen intake between cases and controls.

T05029: A double blind placebo controlled crossover trial of soy phytoestrogens in patients with compensated hypothyroidism

Several problems have been encountered in this ongoing project, including difficulties in patient recruitment and retention as well as problems obtaining soy preparations containing the required amount of isoflavones. Lower isoflavone concentrations were being used than originally planned and it was uncertain how this, together with a lower number of participants, might affect the power of the study. However, it was noted that if the problems could be overcome the anticipated outcomes should help address the Food Standards Agency's policy need of investigating the effects of phytoestrogens on thyroid function in hypothyroid individuals.

T05030: Investigation of the phytoestrogen intake of a group of postmenopausal women previously diagnosed with breast cancer

This ongoing study was considered cost effective, using appropriate methodology. The study should provide sufficient information to assess the phytoestrogen intake of postmenopausal women with breast cancer, a subgroup of the population for which concerns have been raised regarding consumption of large amounts of phytoestrogens. However, from the information available it was unclear how the study population had been selected and therefore how far the results could be extrapolated.

Status of the Phytoestrogen Research Programme

14. A strategic review within the FSA subsequently decided that the scope of the Phytoestrogen Research Programme extended to areas outwith the FSA's remit and that no further research should be funded under this programme. Any future work on the potential risks of phytoestrogens would be commissioned under the Risk Assessment programme (T01).

2011 COT review of Phytoestrogen Research Programme

15. In the current review, the Committee considered outcomes from the studies on-going at the time of the last external review, along with findings from the previous reviews, to evaluate whether the programme had met its objectives and provided the Agency with useful information and value for money.

16. The Committee noted that the three on-going studies were based on the recommendation that the programme concentrate on human studies. There was some lack of clarity in the scientific objectives of the study on phytoestrogen exposure in women diagnosed with breast cancer (T05030), and the Committee was disappointed that the results of this study had not been published in the peer reviewed literature. Further details of the project and a link to the final report can be found at

http://www.food.gov.uk/science/research/foodcomponentsresearch/phytoestrogensresearch/t05-t06programme/t05t06projectlist/t05030/

17. The other two projects had led to a number of publications. However, while both were well designed and conducted, their results were not sufficiently strong to support definitive conclusions.

18. The Committee noted that the analysis of phytoestrogens in a wide range of foods was useful and had allowed robust estimation of short term dietary exposures to phytoestrogens (T05028). While findings indicated no association between phytoestrogen intake and risk of breast cancer, the data on prostate cancer were inconclusive. Further details of the project and a link to the final report can be found at:
http://www.food.gov.uk/science/research/foodcomponentsresearch/phytoestrogensresearch/t05-t06programme/t05t06projectlist/t05028/

19. The Committee had recently considered in detail draft publications on the first and second arms of the study on soy in patients with compensated hypothyroidism (T05029), and had discussed the implications of the results. The project had been significantly delayed by difficulties in recruitment, but despite this, the quality of the work delivered was high. The Committee concluded that the combined results from the first and second arms of the study on potential effects in individuals with compensated hypothyroidism did not provide a sufficiently strong basis for issuing advice on phytoestrogen consumption to patients with compensated hypothyroidism. However, once the third and final arm of the study was completed, consideration should be given to the value of further research to resolve outstanding uncertainties. Further details of the project can be found at:
http://www.food.gov.uk/science/research/foodcomponentsresearch/phytoestrogensresearch/t05-t06programme/t05t06projectlist/t05029/

20. The Committee noted that the research programme had made a significant contribution to the COT report on phytoestrogens published in 2003. The technical difficulties encountered in projects examining biological effects of phytoestrogens *in vitro* and *in vivo* had been addressed appropriately and resulted in work of satisfactory quality. Despite the limitations of small sample sizes, the clinical studies had provided useful information on a number of important endpoints, including several which have not been addressed by other researchers in the UK or internationally.

21. A significant strength of the programme had been the development of analytical standards for a wide range of phytoestrogens. This had enabled the accurate quantitation of phytoestrogens in foods, supplements and biofluids, which had made a number of the clinical and biological studies possible. The standards had been used in FSA-funded projects and continue to be used in phytoestrogen research studies funded by other bodies. This significant contribution extended beyond the original aim of the projects and reflected the quality of the synthetic chemistry applied in producing the compounds. It further demonstrated the benefit of a multidisciplinary programme which facilitated dialogue between different disciplines.

22. Overall the Committee considered that the T05 programme had met its original remit and had delivered work of at least satisfactory scientific quality and in some cases of very high quality. The work had delivered value for money in all cases and in some cases exceptional value for money. The programme had covered areas not addressed elsewhere and helped to reduce uncertainties in the understanding of phytoestrogen effects and exposures. In doing so the programme had assisted in delivering the FSA's policy requirements.

23. The Committee noted that any further work on potential risks associated with phytoestrogens would be funded under the Risk Assessment Research Programme.

24. The Committee was informed that two further projects had been funded to address risks in specific sub-populations and were on-going.

> T01057: A double blind placebo controlled parallel trial of soy phytoestrogens in patients with compensated hypogonadism. Further details can be found at http://www.food.gov.uk/science/research/foodcomponentsresearch/riskassessment/t01programme/t01projlist/t01057/
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> and
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> T01060: A double blind placebo controlled parallel trial of soy isoflavones on markers of bone turnover in females in the early menopause. Further details can be found at http://www.food.gov.uk/science/research/foodcomponentsresearch/riskassessment/t01programme/t01projlist/t01060/

25. The Committee would be informed of the outcome of these studies and might be asked to consider the implications of the results from the studies. Other than the need to consider further research on potential effects in individuals with compensated hypothyroidism to address outstanding uncertainties, the Committee identified no pressing needs for additional research.

COT Statement 2012/01
April 2012

Annex1. List of projects in the Phytoestrogens Research Programme

Project Number	Name	Contractor	Dates
T05001	Synthesis of labelled and unlabelled isoflavonoid phytoestrogen standards	Dr N Botting University of St Andrews	01/05/1996 to 31/05/2002
	A wide range of unlabelled and ^{13}C labelled phytoestrogens and their metabolites were synthesised. These included: • Daidzein, genistein, formononetin, biochanin A, equol, glycitein, O-desmethylangloensin, secoisolariciresinol, matairesinol, enterodiol, enterolactone, 8-prenylnaringenin and 8-prenylgenistein • Sulphated derivatives of daidzein (daidzein-4'-sulphate and daidzein-7,4'-disulphate) and formononetin (formononetin-7-sulphate) • $^{13}C_3$-daidzein, $^{13}C_3$--genistein, ^{13}C-$_3$-secoisolairciresinol, $^{13}C_3$-desmethylangloensin, ^{13}C-$_3$-enterodiol, $^{13}C_3$--matairesinol, $^{13}C_3$--enterolactone, $^{13}C_3$-O-desmethylangloensin These compounds were used in the T05 programme to support numerous analytical and metabolic studies on phytoestrogens. The synthesis of phytoestrogen standards was extended into projects T05023 and T05028.		
T05002	Dietary phytoestrogens; possible beneficial and adverse effects in men	Dr A Collins Rowett Research Institute	01/02/1997 to 30/06/2000
	The phytoestrogens genistein, daidzein, coumestrol and equol were found to inhibit the growth of human prostate cancer cells in culture. In addition, genistein was found to act as an antioxidant, protecting the cells against DNA damage, although it was not as potent as other recognised antioxidants such as vitamins C and E. The antioxidant activity was also evident when 16 healthy men were given tablets containing about 50 mg of phytoestrogens (genistein, daidzein and glycitein) daily for 3 months. However, this treatment produced no changes in hormone (follicle stimulating hormone or testosterone) concentrations or in levels of cholesterol (total, high density lipoprotein or low density lipoprotein). In a separate experiment, there were no effects on sperm number or quality when 14 healthy men were given tablets containing 40 mg of phytoestrogens (genistein, daidzein and glycitein) daily for 2 months. In a final experiment, dietary supplementation with a total of 100 mg of phytoestrogens (genistein, daidzein and glycitein) in tablet form daily for 1 month produced a similar effect on prostate cancer progression in 3 men with the disease as that observed with oestrogen treatment. **Publications** Mitchell, J.H. et al (1998). Antioxidant efficacy of phytoestrogens in chemical and biological model systems. Arch biochem biophys **360**, 142-148. Mitchell, J.H. et al (1999). Effects of a soy milk supplement on plasma cholesterol levels and oxidative DNA damage in men - a pilot study. Eur J Nutr **38**, 143-148. Mitchell, J.H. (2000). Effects of phytoestrogens on growth and DNA integrity in human prostate tumour cell lines: PC-3 and LNCaP. Nutr Cancer **38**, 223-8.		

T05003	Possible effects of dietary phytoestrogens on prostate cancer and 5-alpha reductase activity	Dr F Alexander University of Edinburgh	01/06/1998 to 31/01/2002
	A group of prostate cancer cases (270 men) and a group of appropriate controls (270 men) was selected from a Scottish population. Both current and past phytoestrogen intake were estimated from a food frequency questionnaire, with current intake validated against levels of phytoestrogens measured in the blood. In addition, an attempt was made to measure the activity of 5-alpha-reductase in the blood. Blood phytoestrogen levels were similar in prostate cancer patients and controls, with the exception of enterolactone, levels of which were higher in controls. 5-alpha-reductase activity could not be measured due to technical difficulties. Overall, dietary consumption of phytoestrogens by Scottish men was found to be very low, and unlikely, at such low levels, to influence the risk of prostate cancer materially.		
T05004	Effects of phytoestrogens and related dietary components on bone metabolism	Dr S Robins Rowett Research Institute	01/02/1997 to 31/01/2000
	The effects of dietary supplementation with phytoestrogens on bone density and biological markers of bone formation/resorption were investigated in groups of pre-, peri- and post-menopausal women (200 women in total). Each woman received either a phytoestrogen supplement or placebo treatment daily for one year. In addition, the effects of phytoestrogens on bone cells were investigated in vitro. Dietary supplementation with 40 mg of phytoestrogens (i.e. genistein, daidzein, biochanin A and formononetin) in tablet form daily for one year was found to reduce slightly urinary levels of markers of bone resorption in pre- and peri-menopausal women, but not in post-menopausal women. Phytoestrogen supplementation did not affect bone formation in any of the groups. Genistein inhibited cell growth, induced cell death and interfered with cellular interactions in cultured human bone-forming cells. Genistein also impaired the function of cultured pig cells involved in bone resorption. However, these effects only occurred at very high genistein concentrations (i.e. 10 µM), well above those that might occur in the body following dietary exposure to phytoestrogens. Overall, the results of this project suggest that phytoestrogens may have a weak beneficial influence on bone loss although the significance of this effect, particularly for post-menopausal women, remains to be established. **Publications** Hunter, I. et al (2001). Caspase-dependent cleavage of cadherins and catenins during osteoblast apoptosis. J Bone Miner Res **16**, 466-77.		
T05005	Development and application of screening assays for the beneficial and adverse effects of phytoestrogens in food.	Dr M Sauer Veterinary Laboratories Agency	01/04/1997 to 30/09/2001
	Two biological systems to measure the potency of oestrogenic compounds were developed. The first system was enzyme-based, whereas the second involved the use of yeast cells. Analysis of purified phytoestrogens using the systems showed that		

	phytoestrogens present in soya were 2000-8000 times less potent than the natural human oestrogen, oestradiol. Some phytoestrogens found in hops were more oestrogenic, whereas compounds from onions and citrus fruits were inactive. Analysis of a range of food extracts showed that they were oestrogenic in the following order; a phytoestrogen-containing dietary supplement > soya flour > Burgen bread > soya-based infant milk formula >> white bread (containing soya flour) > cows milk infant formula. The oestrogenic activity of the extracts was found to correlate with the concentrations of phytoestrogens in the samples, which were measured using a chemical analytical method. Some of the phytoestrogens isolated from these extracts were also found to be weakly oestrogenic using a separate bioassay. The biological systems could be used in conjunction with chemical analytical methods to allow improved detection and quantification of phytoestrogens in a wide range of foods. **Publications** Coldham, N.G. et al (2002). A binary screening assay for pro-oestrogens in food: metabolic activation using hepatic microsomes and detection with oestrogen sensitive recombinant yeast cells. Food Addit Contam **19**,1138-47. Coldham, N.G. et al (2001). Identification, quantitation and biological activity of phytoestrogens in a dietary supplement for breast enhancement. Food and Chemical Toxicology **39**, 1211-1224.		
T05006	Investigation of the post-natal developmental toxicity of isoflavones in rats.	Ms S Dawson Astra-Zeneca	01/05/1997 to 01/03/2000
	This project investigated the effects of an isoflavone found in soya-based infant formula on a range of male and female developmental parameters in an experimental model of human neonatal exposure. When administered at a level similar to that given to human infants fed soya-based formulas, the isoflavone genistein had no effects on female sexual development. However, when a dose ten times higher than this was used, female sexual development began at an earlier time-point in the rats. Because of the difference in timing of female sexual development between rats and humans, it is unclear whether higher levels of genistein in soya formulas could alter the timing of female sexual development in humans. . Genistein had no similar effects on male sexual development, even at the higher dose. **Publications** Lewis, R.W., Brooks, N., Milburn, G.M., Soames, A., Stone, S., Hall, M., and Ashby, J. (2003). The effects of the phytoestrogen genistein on the postnatal development of the rat. Toxicol Sci. 71, 74-83.		

T05009	The use of biologically produced ^{13}C enriched isotopomers of the phytoestrogens for use as analytical standards	Dr W Coward MRC - Dunn Nutrition	01/12/1997 to 31/05/2002
	Labelled (^{13}C labels) phytoestrogens were prepared by growing soya beans and linseed in $^{13}CO_2$-enriched air. The phytoestrogens produced by these plants contained ^{13}C instead of ^{12}C. The labelled phytoestrogens were extracted from the plants and purified for use in analytical studies. This project developed methods for the preparation of ^{13}C labelled analytical standards for phytoestrogens. The utility of the labelled phytoestrogens was demonstrated by analysis of food samples by gas chromatography coupled to mass spectrometry.		
T05010	Absorption and metabolism of dietary phytoestrogens in humans - effect of age, gender, food matrix and chemical composition	Prof J Millward University of Surrey	01/07/1998 to 30/09/2003
	Isoflavones were readily absorbed from the gastrointestinal tract and reached maximal concentrations in blood within a few hours of consuming soya foods. Blood and urine levels of phytoestrogens appeared to increase in a non-linear fashion with increased consumption, suggesting that high levels of intake of soya foods may not necessarily result in an equivalent increase of blood and urine phytoestrogen levels. The food matrix affected the absorption of phytoestrogens. They appeared to be absorbed more slowly from solid food (textured vegetable protein) than liquid food (soymilk). In addition, unconjugated phytoestrogens were more bioavailable than conjugated forms. Some gender effects on absorption of phytoestrogens were seen, but the size of this study was not sufficient to establish if these were real differences. No effects of age on phytoestrogen absorption were observed. It was found that there was good correlation between peak blood levels and 24-hour urine levels (urine collected over a period of 24 hours) but poor correlation between spot urine levels (a single urine sample) and blood levels. This is important for future clinical studies, which will require collection of 24-hour urines to monitor isoflavone intake.		
T05011	Influence of human gut microflora on dietary soya isoflavone phytoestrogen bioavailability in adults and children	Dr H Wiseman University of London - KCL	01/10/1998 to 30/09/2001
	This study determined effects of the microflora in the gut on the metabolism of phytoestrogens and levels in the blood and urine of adults and children. The levels of phytoestrogens and their metabolites were measured in the blood, urine and faeces of 76 adults following one of two supervised diets (either high- or low-soya) for 10 weeks. The types of gut microorganisms and chemical processes that alter phytoestrogen availability were also investigated. In addition, babies and children (aged 4 months - 6 years) fed on soya milk formulas in early infancy and equal numbers of age-matched controls fed on cows' milk formula. were recruited. Each group consisted of approximately 6 children in 4 age bands (4-6 months, 6-12 months, 1-3 years, 3-6 years).The levels of phytoestrogens and their metabolites were measured in urine and faecal samples. The types of gut microflora in infants fed soya milk formulas were analysed and compared to those in adults and breast-fed infants. The levels of isoflavones in soya and cows' milk infant formulas were measured. Urine, faeces and blood of the adults on a high soya diet contained the		

	isoflavones genistein and daidzein and their gut metabolites. By comparison the levels of these compounds in the urine, faeces and blood of adults on the low soya diet were very low. Metabolism of the isoflavone daidzein to equol is used as an indication of the presence of certain gut microflora. Around one third of adults have the gut microflora to convert daidzein to equol. Babies fed soya milk formula excreted isoflavones in urine. This shows that they can absorb phytoestrogens from the gastrointestinal tract. Young infants acquired the gut microflora to undertake metabolism of some phytoestrogens. But over time the composition of the gut microflora altered to allow additional metabolic reactions to take place. The type of infant formula consumed (i.e. soya or cows' milk based formulas) influenced the composition and form of the infant gut microflora, which affected the metabolism of isoflavones. **Publications** Hoey, L, Rowland, IR, Llyoyd, AS, Clarke, DB, Wiseman, H (2004). Influence of soya-based infant formula consumption on isoflavone and gut microflora metabolite concentrations in urine and faecal microflora composition and metabolic activity in infants and children. British Journal of Nutrition 91, 607-616. Wiseman H, Casey K, Clarke DB, Barnes KA, Bowey E.(2002) The isoflavone aglycone and gluco-conjugate content of high soy and low soy UK foods used in nutritional studies.. Agric Food Chem.;50:1404-10. Clarke DB, Lloyd AS, Botting N, Needs P, Wiseman H. (2002). Measurement of intact sulphate and glucuronide conjugates of phytoestrogens in human urine using direct injection HPLC and electrospray tandem mass spectrometry.. Anal Biochem. 309:158-72 Wiseman H, Casey K, Clarke DB, Lloyd AS, Rowland IR, Bowey E. (2004). Influence of soy food consumption on plasma, urinary and faecal isoflavone and gut microflora metabolite concentrations, faecal bacterial enzyme activity and conversion of daidzein to equol ex vivo in adult humans. Am J Clin Nutr. 80:692-9		
T05013	Do dietary phytoestrogens protect against cancer in genetically susceptible groups by disrupting metabolism of endogenous estrogens?	Dr C Kirk University of Birmingham	01/10/1999 to 30/09/2002
	The effects of phytoestrogens on oestrogen metabolism were measured in normal and cancerous cells from human breast tissue. A dietary trial investigated the effects of supplementing the diet with soya milk (which is rich in the phytoestrogens genistein and daidzein) for one week on oestrogen metabolism in a group of female volunteers (80 with a genetic susceptibility to breast cancer and 80 controls). Several phytoestrogens were found to inhibit the metabolism of active oestrogen to its inactive form in the human cells. Genistein, daidzein and equol were relatively potent, although the other phytoestrogens only produced effects when present at levels that were much higher than those which would be found in the body from normal dietary intake. Analysis of blood samples showed that consumption of soya milk also inhibited the metabolism of active oestrogen to its inactive form in women from both the group with a genetic susceptibility to breast cancer and the control group. Overall, the results suggest that phytoestrogens might prevent the metabolism of active oestrogen, which is thought to be capable of promoting early breast		

tumours, to its inactive form. However, further work in this area would be required to establish whether this is the case.

Publications:

Kirk, C.J., Harris, R.M., Wood, D.M., Waring, R.H. and Hughes, P.J. (2001). Do dietary phytoestrogens influence susceptibility to hormone-dependent cancer by disrupting metabolism of endogenous oestrogens? Biochemical Society Transactions **29**, 210-216.

Harris, R.M., Wood, D.M., Bottomley, L., Blagg, S., Owen, K., Hughes, P.J., Waring, R.H. and Kirk, C.J. (2004). Phytoestrogens are potent inhibitors of estrogen sulfation: implications for breast cancer risk and treatment. Journal of Clinical Endocrinology & Metabolism **89**, 1779-87.

Kirk, C. J., Bottomley, L., Minican, N., Carpenter, H., Shaw, S., Kohli, N., Winter, M., Taylor, E. W., Waring, R. H., Michelangeli, F. and Harris, R. M. (2003) Environmental endocrine disrupters dysregulate estrogen metabolism and Ca2+ homeostasis in fish and mammals via
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Harris, R.M., Hughes, P.J., Kirk, C.J. and Waring, R.H. (2001) Sulphotransferase inhibition by daidzein and genistein: a role in human health. "Soy & Health 2000" (Eds. K. Desheemaeker & I. Debruyne), Garant Publishers (Leuven - Apeldoorn), 82.

Kirk, C.J., Harris, R.M., Wood, D.M., Hughes, P.J., Anderson, D., Rea, D. and Waring, R.H. (2001) Do dietary phytoestrogens influence susceptibility to breast cancer by inhibiting sulphotransferase enzymes? Abstract to European Commission HEALFO Conference, Lanciano, Italy, Meta srl - Lanciano, pp.82.

Kirk, C.J., Harris, R.M., Wood, D.M., Bottomley, L., Rea, D.W., Hughes, P.J. and Waring, R.H. (2002) Environmental oestrogens: their role in testicular dysgenesis and hormone-dependent cancers. Comparative Biochemistry & Physiology **132**, S2

Harris, R.M., Pettitt, T., Wood, D.M., Bottomley, L., Ayres, S., Rea, D.W., Kirk, C.J. and Waring, R.H. (2004) Plasma levels of free daidzein and genistein are not affected by a high soy milk diet. Cancer Detection and Prevention Prev. **28**, S131-S132.

T05014	The effect of phytoestrogen ingestion on menopausal symptoms	Mr N Bundred University Manchester	01/09/2000 to 30/09/2002
	Fifty postmenopausal women suffering from severe hot flushes consumed either linseed-containing bread buns or placebo bread buns for 3 months before switching to the other type of buns for 3 months. Changes in the level of lignans in the blood were measured, and the effects on hot flushes, hormone levels and biological markers of bone reabsorption / formation analysed. This small pilot study suggested that flaxseed supplementation may have the potential to reduce the number of hot flushes in post-menopausal women. Both placebo and flaxseed supplemented groups experienced a fall in the number of hot flushes during the first three months which suggested a possible placebo effect. However, once women crossed over from placebo to flaxseed supplementation their hot flushes continued to fall in frequency, whereas the incidence of hot flushes rose in the women who changed from flaxseed to placebo supplementation. Lignans (phytoestrogens found in linseed) were measured in the urine and did not appear to have any effect on bone resorption or blood lipids. From the results of this study, flaxseed supplementation reduced the incidence of hot flushes by 60%. Due to the size of the study, further research would be needed to confirm these findings and to determine whether lignans could be used to relieve hot flushes in post-menopausal women.		
T05015	Diet, phytoestrogen and gene nutrient interactions in relation to cancer: a prospective study	Prof S Bingham MRC - Dunn Nutrition	21/03/2000 to 31/03/2003
	Part of the European Prospective Investigation of Cancer (EPIC) study involved a group of 25,630 healthy men and women aged 45-75 years. The volunteers completed food diaries, provided blood samples and were monitored over a number of years to determine the incidence of cancer. Between 1992 and 2002, approximately 200 cases of breast and prostate cancer occurred in this group of people. This project analysed the samples and data collected from the cancer patients and from 200 healthy matched controls in the EPIC study, to investigate possible effects of phytoestrogen consumption on the development of breast and prostate cancer and genetic / hormonal factors that might influence cancer development. Food diaries and blood samples were analysed to determine phytoestrogen intake and blood levels, and this information was used to investigate whether the incidence of breast and prostate cancer correlated with the intake or with blood levels of phytoestrogens. This study developed accurate methods for measuring low exposure levels of phytoestrogens in urine by gas chromatography-mass spectrometry (GC-MS), and in plasma by liquid chromatography-mass spectrometry (LC-MS). There were high correlations between phytoestrogens measured in spot urine samples and phytoestrogens in plasma. The results indicated that increased phytoestrogen intake was associated with an increased risk of breast cancer, although this was only statistically significant for daidzein and equol. The cohort was too small to confirm whether this was a real association or a chance finding and, therefore, a larger study (T5028) was conducted to investigate this further.		

	Publications: Grace, P.B, Taylor, J.I., Low, Y.L., Luben, R.N., Mulligan, A.A., Botting, N.P., Dowsett, M., Welch, A.A., Khaw, K.T., Wareham, N.J., Day, N.E. and Bingham, S.A. (2004) 'Phytoestrogen concentrations in serum and spot urine as biomarkers for dietary phytoestrogen intake and their relation to breast cancer risk in EPIC-Norfolk'. *Cancer Epidemiology Biomarkers and Prevention* 13: 698-709 Low, Y.L., Taylor, J.I., Grace, P.B., Dowsett, W., Scollen, S., Dunning, A.M., Mulligan, A.A., Welch, A.A., Luben, R.N., Khaw, K.T., Day, N.E., Wareham, N.J. and Bingham, S.A. (2004) 'Phytoestrogen exposure correlation with plasma estradiol in postmenopausal women in European Prospective Investigation of Cancer and Nutrition-Norfolk may involve diet-gene interactions'. *Cancer Epidemiology Biomarkers and Prevention* 14: *213-220* Grace, P.B., Taylor, J.I., Botting, N.P., Fryatt, T., Oldfield, M.F. and Bingham, S.A. (2003) 'Quantification of isoflavones and lignans in urine using gas chromatography/mass spectrometry'. *Analytical Biochemistry* 315: 114-21 Grace, P.B., Taylor, J.I., Botting, N.P., Fryatt, T., Oldfield, M.F., Al-Maharik, N. and Bingham, S.A. (2003) 'Quantification of isoflavones and lignans in serum using isotope dilution liquid chromatography/tandem mass spectrometry'. *Rapid Communications in Mass Spectrometry* 17: 1350-1357		
T05016	Effects of phytoestrogens on oestrogen receptor mediated gene transcription, and protein expression	Ms S Dawson Syngenta CTL	21/03/2000 to 31/03/2003
	The effects of oestrogen and one phytoestrogen, genistein, on gene expression were examined in an oestrogen responsive model system (the uterus) and in cultured human cells. In the model system (the uterus), genistein was found to affect the expression of the same set of genes as oestrogen. This suggests that genistein may be able to mimic oestrogen within the body. However, the concentration of genistein used was over 600-fold greater than that of oestrogen (i.e. 250 mg/kg genistein compared to 400 µg/kg oestrogen). Furthermore, the amount of genistein administered was significantly higher than the level that would be attained within the human body from normal dietary intake. Genistein was not found to elicit biological effects through mechanisms unrelated to oestrogenic activity. The results obtained using the model system of the uterus were confirmed using cultured human cells treated with 10^{-5}M genistein and 10^{-8}M oestrogen **Publications**: Murphy, T.C. and Orphanides, G. (2002). Characterisation of the molecular responses to xenoestrogens using gene expression profiling. Phytochemistry Reviews 1, 199-208.		
T05019	The absorption, distribution, metabolism and excretion of isoflavones in vivo	Dr A Cassidy / Prof. J Millward University of Surrey	01/01/1998 to 31/07/2000
	Four human studies involved premenopausal women (n = 10) consuming capsules containing labelled (^{13}C) genistein or daidzein examined the metabolism of a single dose of labelled isoflavones, dose-related changes in phytoestrogen metabolism, effects of ingestion of phytoestrogen-rich foods prior to administration of the labelled isoflavones, and whether the metabolism of daidzein or genistein varied within an individual. The levels of labelled		

	genistein and daidzein were measured in blood, breath, urine and faecal samples. Following ingestion, genistein and daidzein are distributed into a wide range of tissues. Ingestion of 0.8 mg/kg body weight daidzein or genistein led to higher levels in urine and blood than the ingestion of 0.4mg/kg body weight. The concentration of genistein in the blood was higher than the concentration of daidzein, possibly due to more rapid removal of daidzein from the body. Prior consumption of phytoestrogens (50 mg isoflavones from soya milk) for 7 days before ingesting 0.4mg/kg body weight genistein or daidzein did not alter the bioavailability of either phytoestrogen. The urine samples of four of the ten subjects who received the [^{13}C]daidzein doses contained [^{13}C]equol, a metabolite that is produced by micro-organisms in the intestines and which is a more potent oestrogen than daidzein. The times required for levels of genistein and daidzein to reduce by 50% (i.e. their half-lives) were 7.72 hours and 7.75 hours respectively, indicating that isoflavones have a short half-life in the body. Neither genistein nor daidzein could be detected in faecal samples, suggesting that they are not excreted from the body in faeces or are extensively metabolised in the gut. There was good reproducibility in the metabolic fate of genistein and daidzein within individuals, but there were differences between individuals. **Publications** Faughnan, M.S., Hawdon, A., Ah-Singh, E., Brown, J., Millward, D.J. and Cassidy, A. (2004). Urinary isoflavone kinetics: the effect of age, gender, food matrix and chemical composition. Br J Nutr 91,567-74. Setchell, K.D., Faughnan, M.S., Avades, T., Zimmer-Nechemias, L., Brown, N.M., Wolfe, B.E., Brashear, W.T., Desai, P., Oldfield, M.F., Botting, N.P. and Cassidy, A. (2001). Comparing the pharmacokinetics of daidzein and genistein with the use of 13C-labeled tracers in premenopausal women. Am J Clin Nutr 77, 411-9.		
T05020	Absorption, distribution, metabolism and excretion of [^{14}C] labelled genistein	Dr M Sauer Veterinary Laboratories Agency	01/01/1998 to 30/07/2000
	Labelled (^{14}C) genistein was used to examine the tissue distribution, blood concentration and excretion of genistein and its metabolites in rats and whether this differed in males and females. Genistein and its metabolites mainly accumulated in the uterus, vagina, ovary, liver and prostate. The levels of genistein and its metabolites detected were sufficiently high to suggest that they might induce biological effects by interacting with oestrogen receptors found in these tissues. How these findings relate to humans was, however, unknown. Several sex differences in the accumulation, blood concentration and excretion of genistein and its metabolites were identified: In females, genistein was the major residue in the uterus, ovary, vagina and liver. However, in males, the metabolite 4-hydroxyphenyl-2-propionic acid (HPPA) predominated in the prostate, whereas genistein glucuronide was the most abundant residue in the liver. The concentration of genistein and its metabolites in the blood was much lower in females. This may have been due to greater retention by the liver and/or more rapid elimination in females than males. In both males and females, genistein was excreted in urine (67%) and faeces (33%), mainly as metabolites. The major metabolite in males was HPPA, whereas in females the excreted residues were mainly conjugated metabolites of genistein. However, the biological significance of these gender		

	differences remains to be determined. **Publications** Coldham, N.G., Zhang, A.Q., Key, P., Sauer, M.J. (2002). Absolute bioavailability of [14C] genistein in the rat; plasma pharmacokinetics of parent compound, genistein glucuronide and total radioactivity. Eur J Drug Metab Pharmacokinet 27,249-58. Coldham, N.G., Darby, C., Hows, M., King, L.J., Zhang, A.Q., and Sauer, M.J. (2002). Comparative metabolism of genistin by human and rat gut microflora: detection and identification of the end-products of metabolism. Xenobiotica 32, 45-62. Coldham, N.G., Sauer, M.J. (2000). Pharmacokinetics of [(14)C]Genistein in the rat: gender-related differences, potential mechanisms of biological action, and implications for human health. Toxicol Appl Pharmacol. 164, 206-15. Coldham, N.G., Howells, L.C., Santi, A., Montesissa, C., Langlais, C., King, L.J., Macpherson, D.D., Sauer, M.J. (1999). Biotransformation of genistein in the rat: elucidation of metabolite structure by product ion mass fragmentology. J Steroid Biochem Mol Biol 70, 169-84.		
T05021	Quality assurance scheme for phytoestrogens	Central Science Laboratory	01/04/1997 to 31/03/1999
	The accuracy and reproducibility of the different methods used to measure phytoestrogens was determined by assessing the ability of 6 laboratories involved in the Phytoestrogens Research Programme to analyse two phytoestrogens (i.e. genistein and daidzein) in soya flour and urine samples distributed to them. The laboratories' results were found to be reliable and reproducible, although urine analysis produced greater variability in results than soya flour analysis. Genistein was more difficult to measure than Daidzein		
T05022	Biological effects of phytoestrogens	Prof S Bingham MRC - Dunn Nutrition	01/01/1995 to 31/03/1998
	An improved analytical method was developed to measure two phytoestrogens, genistein and daidzein, in food and urine. Analysis involved enzymatic hydrolysis before gas chromatography and mass spectrometry (GC-MS). The method was found to measure phytoestrogens accurately over a wide range of concentrations. Analysis of 274 foods showed that: • 160 foods contained genistein and/or daidzein. The range of concentrations detected varied greatly (i.e. from 0.5 µg/kg to 2000000 µg/kg for the two phytoestrogens combined). • Soya products contained the highest levels (i.e. 0.5 g/kg - 2000000 µg/kg) of the two phytoestrogens. • The remaining 152 foods contained lower amounts of the two phytoestrogens (i.e. 0.5 µg/kg - 8000 µg/kg). For example, cereal products contained concentrations in the upper part of this range, whereas fruit contained levels that varied throughout the range, and some vegetables contained less than 100 µg/kg. Samples were collected as part of the European Prospective Investigation Into		

	Cancer (see T05028). The effects of cooking were examined in 56 foods. In general, boiling in water decreased the concentration of genistein and/or daidzein, although the extent of this decrease depended on the type of food. The investigation of the possible health effects of phytoestrogens (genistein and daidzein), jointly funded with the Medical Research Council, suggested that phytoestrogens might have a protective effect on bone loss in the lumbar (lower) spine in women. However, there were no effects on other parts of the spine or the femoral neck . There were no significant effects on mammographic breast density, hormone levels, menopausal symptoms or markers of cardiovascular disease in the subject group. **Publications** Atkinson, C., Compston, J.E., Day, N.E., Dowsett, M. and Bingham, S.A. (2004). The effects of phytoestrogen isoflavones on bone density in women: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr. 79, 326-33. Atkinson, C., Warren, R.M., Sala, E., Dowsett, M., Dunning, A.M., Healey, C.S., Runswick, S., Day, N.E. and Bingham, S.A. (2004). Red clover-derived isoflavones and mammographic breast density: a double-blind, randomized, placebo-controlled trial. Breast Cancer Res. 6, R170-9. Liggins, J., Mulligan, A., Runswick, S. and Bingham, S.A. (2002) Daidzein and genistein content of cereals. Eu J Clin Nutr. 56, 1-6. Liggins, J., Bluck, L., Coward, W.A., and Bingham, S.A. (1998). Extraction and quantification of daidzein and genistein in food. Anal Biochem. 264, 1-7. Liggins, J., Bluck, L., Coward, W.A. and Bingham, S.A. (1998). A simple method for the extraction and quantification of daidzein and genistein in food using gas chromatography mass spectrometry. Biochem Soc Transact. 26, S87-S88.		
T05023	Synthesis of standard phytoestrogens in labelled and unlabelled form	Dr N Botting University of St Andrews	01/07/2001 to 31/05/2004
	This project developed methods for the preparation of analytical standards for phytoestrogens and their metabolites. The project extended and expanded on the research performed in project T05001. It successfully produced a comprehensive set of ^{13}C-labelled phytoestrogen standards that have been used by other contractors in the T05 programme to undertake metabolic and analytical studies on phytoestrogens A patent between the FSA and the University of St Andrews was agreed to protect the synthetic methods used to make these materials, as well as their application in metabolic and analytical studies. **Publications:** M.F. Oldfield, L. Chen and N.P. Botting, (2004) The synthesis of [3,4,8-$^{13}C_3$]daidzein, Tetrahedron, 60, 1887-1893. N. Al-Maharik and N.P. Botting, (2004) A new short synthesis of coumestrol and its application for the synthesis of [6,6a,11a-$^{13}C_3$]-coumestrol, Tetrahedron, 60, 1637-1642.		

	N. Al-Maharik and N.P. Botting, (2003) Synthesis of lupiwighteone via a para-Claisen-Cope rearrangement, Tetrahedron, 59, 4177-4181. Al-Maharik, N., Botting, N.P. (2006) A facile synthesis of isoflavone 7-O-glucuronides. *Tetrahedron Lett* **47**: 8703-8706		
T05024	Quality assurance scheme for the Phytoestrogen Research Programme	Dr P Finglas BBSRC - Institute of Food Research	01/04/2001 to 31/03/2003
	This project involved an inter-laboratory Quality Assurance Scheme to examine whether the analytical data obtained by contractors undertaking scientific research for the FSA's Phytoestrogens Research Programme were precise and consistent. International laboratories were also able to participate in the scheme. Stable triply labelled (^{13}C) isoflavone standards were provided for participants to use as an internal quality control. Samples of soy infant formula, urine and plasma containing isoflavones were analysed. Participants returned the results for statistical evaluation and each round of testing was reported. After all four rounds, the performance of the laboratories was assessed. The analytical performance of the laboratories measuring daidzein and genistein in the three types of matrix analysed (infant formula, plasma and urine) was found to be acceptable. Suitable methods that perform well for the analysis of daidzein and genistein were identified. **Publications:** Key, E.P., Finglas, P.M., Coldham, N., Botting, N., Oldfield, M.F., Wood, R. (2006) An international quality assurance scheme for the quantitation of daidzein and genistein in food, urine and plasma. *Food Chemistry* **96**: 261-72		
T05025	Effect of ERbeta over expression on molecular action of phytoestrogens	Dr P Darbre University of Reading	01/10/2001 to 28/04/2005
	The effects of ER beta overexpression on the regulation of gene expression by phytoestrogens in breast, uterine and bone cells was examined. Over expression of ER beta reduced induction of CAT (a stably integrated reporter gene) activity by estrogen, daidzein, equol, coumestrol and 8-prenylnaringenin, but enhanced gene expression with high concentrations of genistein and resveratrol. Further work to enhance the over-expression of ER beta would be needed to establish the significance of these findings. **Publications:** Shaw, L.E., Sadler, A.J., Pugazhendhi, D., Darbre, P.D. (2006) Changes in oestrogen receptor-alpha and -beta during progression to acquired resistance to tamoxifen and fulvestrant (Faslodex, ICI 182,780) in MCF7 human breast cancer cells. *J Steroid Biochem Mol Biol* **99**: 19-322 Matsumara, A., Ghosh, A., Pope, G.S. and Dabre, P.D. (2005) 'Comparative study of oestrogenic properties of eight phytoestrogens in MCF7 human breast cancer cells'. *Steroid Biochemistry and Molecular Biology*. 94: 431-443		

T05028	Dietary and biomarker prospective study of phytoestrogens in breast and prostate cancer	Prof S Bingham MRC - Dunn Nutrition	01/10/2004 to 30/09/2007
	A rapid and sensitive method for the analysis of phytoestrogens in foods was developed using liquid chromatography coupled with mass spectrometry (LC/MS) and automated solid phase extraction. Once the method was established, 509 foods were analysed for phytoestrogen content and the values incorporated into an established nutritional prospective study data base of diet and cancer, known as the European Prospective Investigation of Cancer (EPIC). The dietary intake of phytoestrogens, and biomarkers of intake in blood and urine samples, were related to the incidence of breast and prostate cancer in the EPIC study. It was established that only 5% of 509 foods commonly consumed in the UK contain more than 700 µg phytoestrogen per 100 g wet weight. For the first time, the phytoestrogen content of foods of animal origin was determined. Meat, fish, seafood, eggs and dairy products were all found to contain phytoestrogens, with an average content of 20 µg per 100 g. Of particular note was the difference in phytoestrogen content observed in soya-based infant formula; which was found to be 300 times greater than that of traditional infant formula (19200 µg/100 g vs. 59 µg/100 g). The study established that preparation and cooking can have a marked effect on the phytoestrogen content of food. It revealed that the phytoestrogen content of fruit and vegetables decreases significantly when they are boiled (as the phytoestrogens leach into the discarded water). It was also noted that peeling fruit and vegetables reduces their phytoestrogen content. No relationship was found between the dietary intake of phytoestrogens and the risk of breast or prostate cancer amongst individuals in the EPIC study; with the mean phytoestrogen intake found to be similar between controls and cases of breast and prostate cancer. **Publications**: Kuhnle GG, Dell'aquila C, Aspinall SM, Runswick SA, Mulligan AA, Bingham SA. **(**2008**)** Phytoestrogen Content of Beverages, Nuts, Seeds, and Oils *J. Agric. Food Chem. 56:* 7311–7315 Kuhnle GG, Dell'aquila C, Aspinall SM, Runswick SA, Mulligan AA, Bingham SA (**2008)** Phytoestrogen Content of Foods of Animal Origin: Dairy Products, Eggs, Meat, Fish, and Seafood *J. Agric. Food Chem. 56:*10099–10104 Kuhnle GG, Dell'aquila C, Aspinall SM, Runswick SA, Mulligan AA, Bingham SA. (2009) Phytoestrogen content of cereals and cereal-based foods consumed in the UK Nutr Cancer.;61:302-9 Ward HA, Kuhnle GG, Mulligan AA, Lentjes MA, Luben RN, Khaw KT. (2010) Breast, colorectal, and prostate cancer risk in the European Prospective Investigation into Cancer and Nutrition-Norfolk in relation to phytoestrogen intake derived from an improved database. Am J Clin Nutr. 91:440-8 Kuhnle GG, Ward HA, Vogiatzoglou A, Luben RN, Mulligan A, Wareham NJ, Forouhi NG, Khaw KT. (2011) Association between dietary phyto-oestrogens and bone density in men and postmenopausal women. Br J Nutr. 106:1063-9		

T05029	A double blind placebo controlled crossover trial of soy phytoestrogens in patients with compensated hypothyroidism	Dr S Atkin University of Hull	03/01/2005 to 30/09/2012
	In a double blind, placebo-controlled, crossover trial, 134 patients with compensated hypothyroidism were given either a soya protein preparation that was isoflavone free or a preparation with a known concentration of soya isoflavones. Clinical effects and any changes in thyroid status were determined by analysis of blood and urine biochemistry. This research assessed whether soya has clinically important effects in patients with compromised thyroid function. **Publications**: Sathyapalan, T., Manuchehri, A.M., Thatcher, N.J., Rigby, A,S., Kilpatrick, E.S., Chapman, T. & Atkin, S.L. (2010) The effect of soy phytotestrogen supplementation on thyroid status and cardiovascular risk markers in patients with subclinical hypothyroidism: a randomized double blind crossover study. *The Journal of Clinical Endocrinology & Metabolism*, published online doi: 10.1210/jc.2010-2255		
T05030	Investigation of the phytoestrogen intake of a group of post menopausal women previously diagnosed with breast cancer	Mr R Rainsbury Winchester & Eastleigh NHS Trust	01/09/2004 to 31/08/2006
	Post-menopausal women participating in the Women's Intervention Nutrition Study (UK) were included in the project. The women completed 4-day food diaries and these were used to estimate intake of specific phytoestrogens. Additional intake from supplements was determined by laboratory analysis of the supplements. Systemic exposure to phytoestrogens was also assessed in a sub-group of women by means of a 24-hour urine collection. Women treated with anti-oestrogenic drugs, such as tamoxifen, tended to have higher higher average phytoestrogen intakes but there was no relationship between the oestrogen receptor status of the women and levels of phytoestrogen intake. This study developed a more complete dietary analysis database for determining the intake of phytoestrogens. This database should be a valuable resource for future studies on phytoestrogen exposure. There was considerable variation in phytoestrogen intake between the 316 women's diaries analysed, reflecting individual food preferences, limitations of the dietary analysis database, and variations in the women's existing levels of information and awareness about phytoestrogens combined with a lack of routine access to dietary information. This variation was also seen in the urine analysis, which confirmed the validity of the food diary as a tool for measuring phytoestrogen intake. The study identified a gap in the routine availability of evidence-based dietary information for breast cancer patients. For most women, having breast cancer had not changed their diet. In part, this was because they had received limited or no advice at the point of diagnosis on a possible connection between diet or nutrition and cancer. As a result of this lack of information they relied upon their own understanding of healthy food and common sense, with other factors such as needs of family members and cooking on a budget having a stronger influence than cancer diagnosis.		

T06001	Identification and quantification of dietary lignans by liquid chromatography and mass spectrometry	Dr M Sauer. Veterinary Laboratories Agency	01/04/1999 to 01/09/2001
	The oestrogenic/anti-oestrogenic activity of selected plant- and microflora-derived lignans was examined. The production of lignans by gut microflora was investigated. An improved analytical method (involving liquid chromatography with tandem mass spectrometry (LC-MS/MS)) was developed, validated and used to measure the concentration of lignans in a range of foods and in the milk of cattle fed a lignan-rich diet. The amount of lignans released from food samples by gut microflora and by acid hydrolysis was compared. The plant lignans secoisolariciresinol (SIL) and matairesinol (MIR) are converted in the body to the metabolites, enterolactone (EL) and enterodiol (ED). All of these compounds were found to have low oestrogenic or anti-oestrogenic potency. Lignans may therefore act only very weakly on processes involving oestrogen within the body, although it is possible that they act through other mechanisms (e.g. as antioxidants). Human faecal microflora were found to convert SIL to ED, and to convert MIR to a complex range of unidentified metabolites. For all foods tested, digestion with microflora released either an equivalent or greater amount of total lignans than acid hydrolysis, which is normally used to extract lignans from food prior to analysis. Release of lignans from foods by microfloral digestion was considered to provide a more accurate representation of total lignans likely to be released from food in the gut. An improved LC-MS-MS method for the analysis of phytoestrogens was developed and validated. Analysis of 13 types of food showed that their lignan content ranged widely, as follows: linseed (~6,000 µg/g) >> Burgen bread (~400 µg/g) >> leaf tea > rye bread > carrot, banana, red wine, lentils, cheese > full fat cream, skimmed milk, yoghurt (0.04 ng/ml). Milk from cattle fed linseed-rich diets was found to contain similar amounts of lignans to milk from cattle fed standard diets, indicating that transfer of lignans to milk is inefficient. **Publications** Coldham, N.G. and Sauer, M.J. (2001). Identification, quantification and biological activity of phytoestrogens in a dietary supplement for breast enhancement. Food and Chemical Toxicology **39**, 1211-1224.		
T06002	Phytoestrogen dietary supplement survey	Dr M Sauer . Veterinary Laboratories Agency	01/04/1999 to 01/03/2001
	A range of phytoestrogens was quantified in 50 soya-based dietary supplements. Fifteen of these products were also tested for oestrogenic activity. The relative risk associated with the recommended intake of the supplements was evaluated by comparing their phytoestrogen content and oestrogenic activity with that of commonly available foods including soya flour and soya-based infant formulas. An analytical method (Liquid chromatography coupled to tandem-mass spectrometers [LC-MS/MS]) was developed to identify and measure the concentrations of phytoestrogens in 50 dietary supplements. The phytoestrogen content of the supplements varied widely between products both in terms of total phytoestrogen present and the total oestrogenic potential. Many supplements contained lower amounts of phytoestrogens than stated on the product labels.		

	Publications Coldham, N.G. and Sauer, M.J. (2001). Identification, quantitation and biological activity of phytoestrogens in a dietary supplement for breast enhancement. Food and Chemical Toxicology. **39**, 1211-1224. Sauer M. J., Hows, M and Coldham, N. G. Phytoestrogen content of dietary supplements. (in preparation).		
T06003/4	Measurement of phytoestrogens in the UK diet	Dr. Massey. Central Science Laboratory	01/05/1997 to 01/02/2001
	This project was divided into three stages. The first analysed the levels of phytoestrogens in the diets of 101 vegetarians. The second investigated the phytoestrogen content of a range of foods by analysing 195 Total Diet Study samples, 10 real ales and 14 additional fish samples. The third attempted to identify components of the UK diet containing novel conjugates of the phytoestrogen genistein. Analysis was by liquid chromatography with ultraviolet detection and mass spectrometry (LC-UV-MS). The results of the three stages of analysis undertaken were as follows: <u>Stage 1 (Vegetarian diets)</u> The average (mean) total isoflavone content of the vegetarian diets was 5.4 mg/kg wet weight of food. This was similar in the summer and winter. Most of the diets were based on soya, which contains high levels of isoflavones. However, some of the diets were not soya-based, and these contained isoflavones from other dietary sources. Coumestrol was not detected in any of the vegetarian diets. This indicates that the diets contained little or no legume sprouts (e.g. clover and alfalfa). <u>Stage 2 (Total Diet Study samples)</u> 195 Total Diet Study samples were analysed, which included foods such as fruit, vegetables, dairy products, meat products, cereals and bread. Important sources of isoflavones were meat products and bread, which contained up to 22 and 52 mg/kg wet weight respectively. All of the real ale samples contained at least one isoflavone, as well as relatively high levels of prenylnaringenins (e.g. mean concentrations of 210 µg/l of 6-prenylnaringinen and 61 µg/l of 8-prenylnaringenin). This suggests that for some individuals, real ales may be a significant dietary source of one type of phytoestrogen. Isoflavones were not detected in farmed or ocean fish fillets. However, some fish-based products were found to contain isoflavones (up to 8 mg/kg wet weight), possibly as a result of the addition of isoflavone-containing ingredients during manufacture. For example, the presence of isoflavones in seafood sticks may have been due to inclusion of soya. <u>Stage 3 (Novel genistein conjugates)</u> Chilli powder, curry powder, crushed chilli peppers, tandoori powder and garam masala were identified as significant dietary sources of unusual genistein conjugates. A number of genistein conjugates were detected in food for the first time. One of the vegetarian diets contained genistein-4'-O-glucoside. Chilli extract contained genistein-'4'-(6"-O-acetylglucose). **Publications** Clarke, D.B. and Lloyd, A.S. (2004). Dietary exposure estimates of isoflavones from the 1998 UK Total Diet Study. Food Addit Contam. 21, 305-316.		