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

DRAFT STATEMENT ON REVIEW OF THE PHYTOESTROGENS RESEARCH PROGRAMME – T05

1. The Committee considered a review of the T05 Research Programme at a previous meeting and agreed to produce a statement summarising the contribution of the programme overall and of the final projects commissioned under the programme but not finalised when it was last reviewed in 2007.

2. A draft statement is attached at Annex 1.

Questions asked of the Committee

3. Members are invited to comment on the draft statement on the Phytoestrogens Research Programme at Annex 1.

Secretariat

August 2011.

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

DRAFT STATEMENT ON REVIEW OF THE PHYTOESTROGENS RESEARCH PROGRAMME – T05

This Annex contains a copy of the second draft COT statement on the T05 Phytoestrogen research programme.

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DRAFT STATEMENT ON REVIEW OF THE PHYTOESTROGENS RESEARCH PROGRAMME – T05

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. As a result, concern has been expressed that consuming phytoestrogens might have oestrogenic, anti-oestrogenic and/or other effects in humans. These effects could be either adverse or beneficial and could affect particular subgroups of the population.

2. The T05 Phytoestrogen research programme was established in 1997 by the Ministry of Agriculture, Fisheries and Food (MAFF) to improve the assessment of the human health implications (risks and benefits) of dietary phytoestrogens in order to underpin appropriate information to consumers. The programme's specific remit was to provide answers to 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
- Possible beneficial and/or detrimental effects of phytoestrogens in the general population and in specific (genetic and/or age) subgroups within the population

3. Responsibility for the T05 Phytoestrogen research programme transferred to the Food Standards Agency in 2000.

2000-2001 consensus review of the phytoestrogens programme

4. In 2000-2001, the Food Standards Agency commissioned a consensus review of the phytoestrogens programme, together with a survey of phytoestrogen research being conducted worldwide in order to assess the Agency-funded research in the context of global efforts. The results of this project were evaluated by the Committee on Toxicity (COT) as part of its review on phytoestrogens and health. http://www.food.gov.uk/science/research/foodcomponentsresearch/phytoestrogensresearch/t05-t06programme/t05t06projectlist/t05018project/

5. The reviewers concluded that the programme represented a Network of Excellence in terms of global research into phytoestrogens. Research highlighted as being of particular value were those projects devoted to the synthesis of phytoestrogen standards and the use of transgenic mouse models containing reporter genes.

6. Dividing the phytoestrogens research into six key areas, the reviewers made the following recommendations for future research in the T05 programme:

Analytical and Chemistry

7. 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

8. 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.

Absorption, distribution, metabolism and excretion

9. 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 symptomalogy is an important issue to pursue. Drug-phytoestrogen interaction in transport processes in the liver and kidney should be investigated in human studies.

Mechanisms

10. 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

11. 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

12. 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.

13. The majority of projects in the T05 programme were ongoing, completed or recently commissioned at the time of the consensus review.

2003 COT report on Phytoestrogens.

14. During 2000-2003, a COT working group reviewed the available scientific literature, the research funded in the T05 programme and the results of an external review of the T05 research programme conducted in 2001. A COT report, including recommendations for further research, was published in 2003 and can be found at http://cot.food.gov.uk/cotreports/cotwgreports/phytoestrogensandhealthcot

15. The report recommended future research to address important outstanding issues and to aid future risk assessment of dietary phytoestrogens. The report considered 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.

16. As a result of these recommendations the Food Standards Agency commissioned three further research projects under the T05 programme, 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). Elements highlighted for consideration in the 2001 review were incorporated 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 T05 Programme Review

17. These three projects were ongoing when the T05 Research Programme was subject to a further external review in 2007, together with the FSA T01 Risk Assessment Research Programme. The review panel which comprised 14 independent experts with relevant experience in toxicology and/or a wide range of specific research areas, considered the relative strengths and weaknesses of the T05 programme on the basis of the projects that had been presented.

18. This review included the three new projects, together with those that were at an early stage at the time of the previous review. The general discussion from the Programme Review is quoted in paragraphs 18-21 below, with points made on

individual projects in paragraphs 22-29. The complete report from the Programme Review can be found at:
http://www.food.gov.uk/science/research/foodcomponentsresearch/riskassessment/t01t05t09review/

General discussion

19. 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.

20. 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.

21. 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.

22. 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

23. 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

24. Although transgenic cell lines and mouse strains had been developed as set out in the Scope of Work, 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.

T05023: Synthesis of standard phytoestrogens in labelled and unlabelled form

25. 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

26. 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

27. 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

28. 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

29. 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

30. 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 TO5 Research Programme.

31. A strategic review within the Food Standards Agency subsequently decided that the scope of the T05 programme covered areas outwith the Agency's remit and that no further research would 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 TO5 Research Programme.

32. The Committee considered the outcomes from the studies on-going at the time of the last external review in combination with the previous external reviews to review whether the programme had met its objectives and provided the Agency with valuable information, as well as value for money.

33. The Committee considered that these three studies were based on the recommendation that the programme concentrate on human studies. Although

concerned at the lack of clarity in the scientific objectives in the study on phytoestrogen exposure in women diagnosed with breast cancer, the Committee were disappointed that the results of this study although available on Foodbase 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/

34. The other two projects had lead to a number of publications, however whilst both were well designed and conducted studies the results were insufficient to reach definitive conclusions. 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. Whilst the findings indicated no association between phytoestrogen intake and breast cancer risk, 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/

35. The Committee had recently considered in detail draft publications on the first and second arms of this study and the implications of these results. The Committee concluded that the combined results of 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 had been completed, consideration would need to 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/

36. The Committee noted that the programme had made a significant contribution to the COT report on phytooestrogens 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 appropriate quality. Despite the limitations of small sample sizes, the clinical studies had assessed effects on a number of important endpoints, including several which have not been addressed by other researchers in the UK or internationally.

37. 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 which had made a number of the clinical and biological studies possible. The standards have been used in Food Standards Agency-funded projects and continued to be used in phytoestrogen research studies funded by other bodies.

38. The Committee noted that any further work on potential risks associated with phytoestrogens would be funded under the T01 Risk Assessment Research Programme. The Committee noted that two further projects had been funded to address risks in specific sub-populations and were on-going under this programme.

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/

and

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/

39. The Committee would be informed of the outcome of these studies and may 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 reduce uncertainties, the Committee identified no further pressing research needs.