

Food Research Programmes Annual Report 1999–2000

This report gives details of the progress made on the research funded by the Joint Food Safety and Standards Group in 1999–2000. The continuing projects in the report transferred to the Food Standards Agency on vesting day: 1 April 2000.

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NB: Codes in brackets denote the new Food Standards Agency codes for these programmes.

Introduction

The Food Standards Agency

The main objective of Food Standards Agency is to “protect public health from risks which may arise in connection with the consumption of food, and otherwise to protect the interests of consumers in relation to food”. It became a legal entity on 3 April 2000 following the establishment of Food Standards Act (November 1999). The Agency UK Headquarters are in London with executive bodies in Scotland, Wales and Northern Ireland. To enable it to meet its main functions the UK HQ is divided into five main groups, each containing a number of divisions and these are detailed in Figure 1.

These functions are to:

- provide advice to the public and to Government on food safety, nutrition and diet
- protect consumers through effective enforcement and monitoring
- support consumer choice through accurate and meaningful labelling

The Agency commissions research to support these functions and help ensure that its policies and advice is based on the best available science.

The Joint Food Safety and Standards Group

The research outlined in this document was commissioned before the Food Standards Agency was formed. The research was funded by the Ministry of Agriculture, Fisheries and Food and the Department of Health. Moreover, the programmes that are discussed are continuing in the Agency. Prior to the formation of the Food Standards Agency, the Joint Food Safety and Standards Group (JFSSG) was responsible for commissioning, managing and evaluating research into food safety and standards. JFSSG was a joint body, comprising of officials from Ministry of Agriculture, Fisheries and Food (MAFF) and Department of Health (DH) staff, its remit was to advise Ministers on all matters relating to food safety and standards. It was set up in 1 September 1997 to ensure that there was a seamless transition from MAFF/DH into the Food Standards Agency. For the financial year 1999/2000 the total JFSSG budget for research was ca £21.5 million (Figure 2 shows a breakdown of these funds).

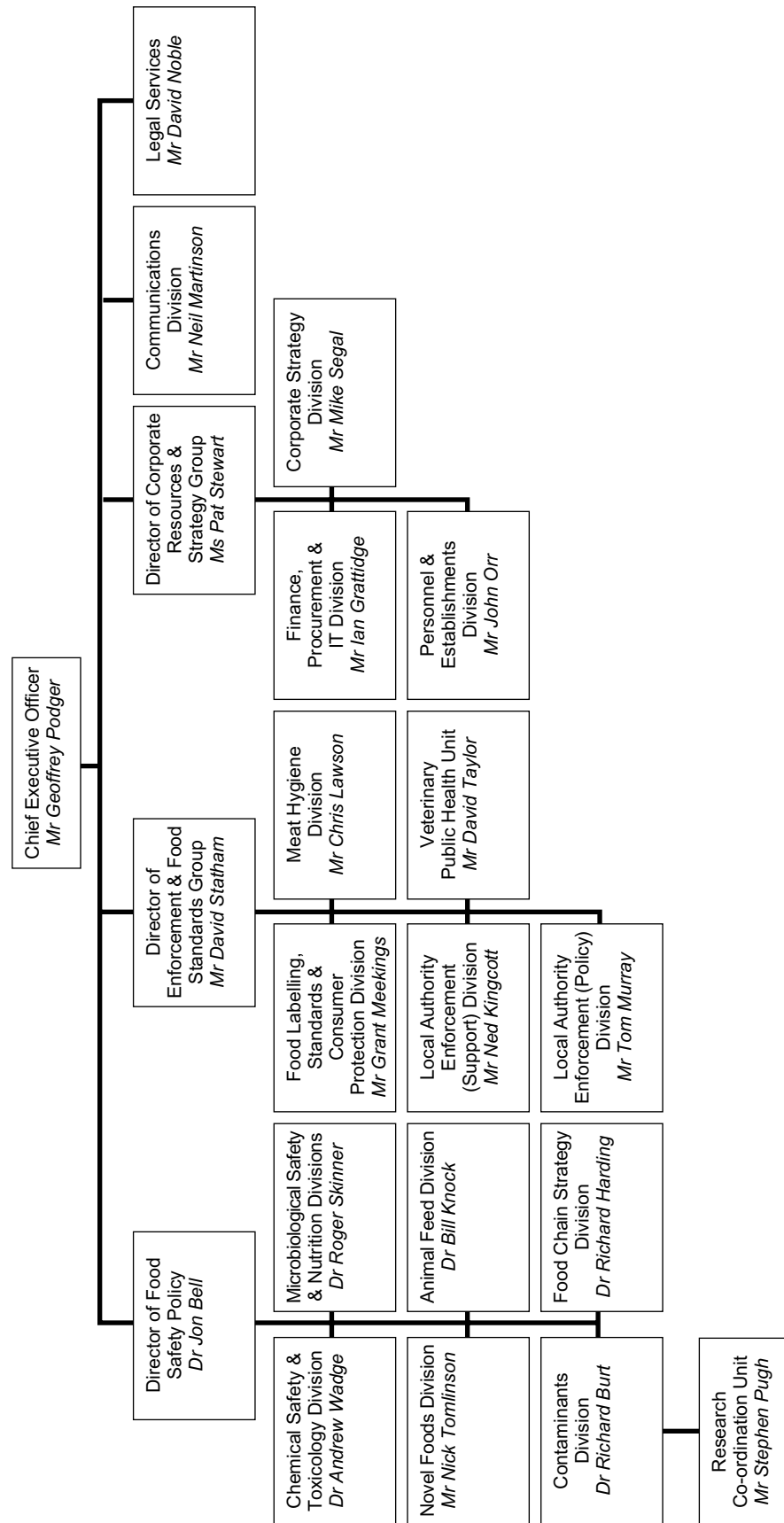
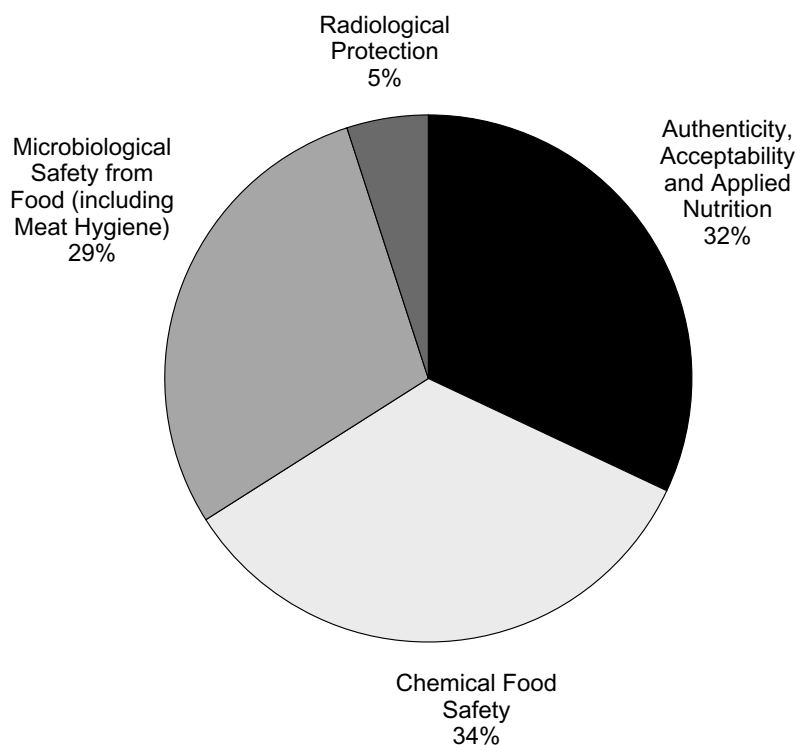


Figure 1: Food Standards Agency – UK Headquarters

Research in the Food Standards Agency

The Food Standards Agency funds research into food safety (which covers three key areas: food poisoning bacteria, chemical contaminants in food and

Figure 2: Division of JFSSG food safety and related budget for 1999/2000



the physiological and biological effects of various food components), food quality and nutrition. These areas are divided into a series of discreet programmes focussing on specific issues that require addressing by the Agency, with each programme having a lifetime of approximately five years.

Research projects are commissioned to answer specific questions within each of the programmes in order that each programme meets its overall objectives. The majority of research projects are funded through open competition and as part of the Agency's commitment to be open and fair; this is likely to continue. In practice this is achieved by the issuing of research requirements each year¹ for each of the programmes that the Agency funds. Proposals received in response to these calls are appraised using *ad hoc* panels. These panels consist of both Food Standards Agency officials and external experts. They evaluate the resulting proposals to ensure that only the proposals most likely to answer the Agency's questions are funded.

¹ In 2000 the Food Standards Agency will issue two Research Requirements Documents with a view to making this a quarterly process in future years.

The high level of relevant scientific expertise within the Food Standards Agency enables individual Policy Divisions to manage the research projects that they commission, either internally or with the aid of external Programme Advisors. To enable the Agency to achieve the best value-for-money for every project that is funded, individual contractors are monitored against a series of milestones set out in their contracts. In addition, contractors are encouraged to discuss progress with Agency staff on a regular basis throughout the lifetime of a project. Upon completion, final reports for each project are evaluated against strict criteria and the conclusions resulting from the work are used to underpin future policy decisions.

Future Research in the Food Standards Agency

During the next 12 months, the Food Standards Agency will be embarking on an extensive review of its research strategy. This will be carried out by a Group chaired by Sir John Arbuthnott comprising of senior Agency officials and experts from consumer organisations, academia, the food industry and other funders of food research. The Review Group will:

- Consider how to ensure that the Agency's research strategy is consistent with its overall strategy;
- Review the current arrangements for openness and consultation on research issues; and
- Review the Agency's overall research programme.

The results of its findings will be used to shape the Food Standards Agency's future research policy.

More information on research and many other aspects of the Food Standards Agency can be obtained from the Agency's website: www.foodstandards.gov.uk.

Food Quality and Applied Nutrition (MINIM PP1:08)

AN02 (now N02) – The role of dietary lipids in the development of cardiovascular disease

1. Background

Fat is an essential nutrient in the human diet and provides energy, essential fatty acids and fat soluble nutrients such as vitamin E, A and D. However, both the total amount and type of fat (lipid) in the diet have been implicated in the causation and/or progression of a number of different health problems including cardiovascular disease (CVD). The Dietary Lipids Programme is concerned with the effect of dietary lipids including triglyceride structure and different fatty acids on the development of cardiovascular disease. The programme is examining the various hypotheses that have been put forward suggesting changes in the quality as well as the quantity of dietary fat could reduce the prevalence of CVD. These include the effect of lowering total fat intake, of changing the ratio of saturated fat to unsaturated fat and of changing the ratio of different types of unsaturated fatty acids (n3:n6).

The aim of the Dietary Lipids Programme is to provide sound scientific evidence on the biological effects of lipids which can be used in the formulation of healthy eating recommendations for consumers. The Dietary Lipids programme was reviewed in 1996 and was again the subject of a review in 2000 which concluded that these projects have contributed substantially to research in the area.

The cost for this programme in Financial Year 1999/2000 was ca. £1.3 million

2. Outputs

n-3 Polyunsaturated Fatty Acids (n-3 PUFA)

In 1996-97, the Research Requirements posed the question:

“What are the relative effects of increasing the intake of long chain n-3 PUFA and altering the ratio of other fatty acids in the diet on the regulation of vasomotor tone, nitric oxide metabolism and endothelial function?”

A number of intervention studies were funded predominantly exploring the relationship between long chain n-3 PUFA and various risk factors for CVD. Long chain n-3 PUFA found in high concentrations in oil rich fish, have been shown to reduce the risk of CVD. Key findings from some of the studies funded so far are:

- Increased plasma and red cell n-3 PUFA through increased fish intake in certain high risk groups for CVD, was related to increased flow mediated dilation in the brachial artery, which is a measure of vascular function and improved circulation.
- Increasing intakes of long chain n-3 PUFA resulted in increased levels of n-3 PUFA in red cell membranes and this was not associated with a decrease in plasma vitamin E concentrations which is known to protect against lipid peroxidation.
- Increasing plasma n-3 PUFA levels were associated with increased HDL and decreased plasma triglyceride concentrations.
- Fish oil supplementation significantly improved endothelium-dependent vascular responses and reduced the level of thrombomodulin (a marker of endothelial damage) in normal subjects.

Decreased sensitivity to insulin is associated with adverse changes in plasma lipid profile, leading to an increase in risk of cardiovascular disease. An intervention study investigating the influence of increased dietary n-3 PUFA and reduced saturated fatty acids (SFA) on insulin sensitivity and post-prandial metabolism of macronutrients showed:

- Long chain n-3 PUFA lowered fasting and post-prandial insulin and triglyceride levels, had a weak activating effect on lipoprotein lipase, lowered fasting non esterified fatty acids and raised HDL cholesterol
- Replacing SFA with monounsaturated fatty acids (MUFA) also improved insulin sensitivity, even in the context of a relatively high fat diet.

3. Dissemination Activity

Results from the projects in the Dietary Lipids programme have been published in internationally recognised journals and presented in national and international meetings and conferences. An independent review of the Lipids Programme was completed in March 2000 and evaluated the results

from the programme over the last 10 years, with special emphasis on results obtained since 1996. This review also included two successful workshops which made 12 recommendations on priority areas, to inform the next ROAME Review of the programme in 2001.

AN02 (now N02) – The role of dietary lipids in the development of cardiovascular disease

Project Code	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0228 (N02001)	Influence of increased dietary n3 PUFA reduced SFA on the insulin sensitivity of postprandial macronutrient utilisation	Surrey University	01/07/95	30/06/99	11,724
AN0233 (N/A)	Evaluation of the effects of dietary supplements of individual fatty acids on vascular tone and endothelial function	Dundee University	01/07/96	30/06/99	70,553
AN0234 (N02002)	Dietary fatty acids and endothelial functions in the control of vasomotor tone and nitric oxide metabolism	Royal Free Hospital School of Medicine	01/07/96	30/06/99	60,234
AN0238 (No2003)	The effect of dietary n-3 and n-6 poly-unsaturated fatty acid intake on atheromatous plaque lipid composition	Southampton University	01/03/97	30/11/00	72,377
AN0240 (N02004)	Optimal intake of MUFAs in the UK diet and the mechanism of their effects on human lipoprotein structure and metabolism	IFR, Institute of Food Research	01/04/97	31/05/01	139,660
AN0240 (N02005)	Optimal intake of MUFAs in the UK diet and the mechanism of their effects on human lipoprotein structure and metabolism	Glasgow Royal Infirmary	01/04/97	31/05/01	100,219

Project Code	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
ANN0241 (N02006)	Effects of dietary substitution of saturated fatty acids with monounsaturated fatty acids	Reading University	01/07/97	30/10/00	190,828
AN0243 (N02007)	Effect of diet on chain elongation and desaturation of alpha-linolenic acid in man	Southampton University	01/03/98	28/02/01	158,432
AN0244 (N02008)	Importance of α -linolenic acid as a source of long-chain n-3 PUFA & its influence on risk factors of cardiovascular disease	Surrey University	01/08/98	31/01/02	109,101
AN0245 (N02009)	Relationship of n-3 fatty acid status to vascular endothelial function & risk factors for coronary heart disease	University of London, Institute of Child Health	01/01/99	31/12/99	37,726
AN0246 (N02010)	Double-blind controlled trial of dietary enrichment with α -linolenic acid on vascular endothelial function in humans	University College of Wales, Medical School	01/11/98	31/12/00	107,304
AN0248 (N02011)	Dietary omega-6 to omega-3 PUFA ratio in UK Asians: relevance to cardiovascular disease risk & modification by dietary means	Reading University	01/07/99	30/06/02	102,610
AN0249 (N02012)	Establish a novel centrifugation method to separate human plasma lipoproteins on self generating gradients	Sheffield University	01/06/99	31/09/01	51,250

Project Code	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0250 (N02013)	Evaluate effects of dietary exchange of individual saturated fatty acids on haemostasis & vascular function	Dundee University	01/09/99	31/08/02	68,512
AN0251 (N02014)	A consensus review of the MAFF Lipids Programme	Ashwell Associates	01/04/99	31/03/00	55,106
TOTAL					1,335,636

AN03 (now N03) – The role of complex carbohydrates in the diet

1. Background

Increased consumption of complex carbohydrates is generally accepted as likely to be associated with public health benefit for three reasons. First, increasing the complex carbohydrate component of the diet tends to reduce the fat content with consequent advantages in the reduction of both cardiovascular disease risk and the development of obesity. Secondly, there is improved insulin sensitivity, reduction of which is a known risk factor for coronary heart disease and possibly diabetes. Thirdly, there is an increase in the amount of carbohydrate that resists digestion in the small intestine and thus becomes available for fermentation by bacteria in the colon. Such fermentation is known to increase the amount of biomass and thus aid intestinal transport (reduce constipation) but there is evidence that the products of fermentation (short chain fatty acids – SCFA) could be beneficial to the colonic mucosa. The Committee on Medical Aspects of Food and Nutrition Policy Report on Nutritional Aspects of the Development of Cancer recommended an increase in intake of NSPs from a variety of food sources on the basis of moderately consistent evidence that higher intakes would reduce the risk of colo-rectal cancers.

The total spend for this programme in 1999/2000 was *ca.* £ 280k.

2. Outputs

The results from the projects in this programme have demonstrated that complex carbohydrates play an important role in the provision of fermentable substrate to the colonic microflora and, via the products of fermentation, on mucosal homeostasis and function. The programme has shown that complex carbohydrates have little effect on insulin sensitivity whereas fat intake has a substantial effect.

Research funded through the AN03 (now N03) research programme has demonstrated that some forms of complex carbohydrates (non-starch polysaccharides – NSP) can help to sequester potential cancer-causing chemicals, thereby preventing contact with the colonic epithelium. Other AN03 research has shown that the principal fermentable materials in the colon are mucin (derived from the small intestine itself), resistant starch and NSP. These are associated with increased cell proliferation which is not mediated by enteroglucagon. The consequences of increased cell division induced by resistant starch and NSP are being investigated in ongoing research projects.

Initial results from a study into potential markers for carbohydrate intake were reported last year. The findings suggested that insulin-like growth factor-1 (IGF-1) and its binding protein IGFBP-1 are not convincing markers for carbohydrate intake. Although findings from the study suggested that IGFBP-1 may be a marker for impaired glucose tolerance, insulin resistance and an adverse cardiovascular risk profile. The project also developed a food frequency questionnaire for use in the Pakistani Muslim population. Additionally, a database of recipes from the Pakistani Muslim population was collected and analysed for nutrient content.

A project investigating the effect of increased consumption of complex carbohydrates on micronutrient availability was completed last year. The association between nutrient intakes of carotenoids, vitamin A, vitamin E, thiamin, riboflavin, vitamin B6, vitamin B12, folic acid, vitamin C and trace elements and plasma nutrient levels was assessed taking into account non-starch polysaccharide (NSP) intakes and other dietary and lifestyle factors. The results suggested that higher levels of NSP did not significantly inhibit absorption of any micronutrients but were associated with higher levels of carotenoids and ascorbic acid in the plasma.

3. Dissemination Activity

The results of work supported within this programme have been published by individual contractors in the scientific literature.

4. Future Activity

The 1996 Review of the Nutrition R & D programmes concluded that no further work should be commissioned under the programme until results from all on-going projects are available.

The Programme Manager in 1999/2000 was Ms Helen Lee, Nutrition Unit, MAFF (now Food Standards Agency).

AN03 (now N03) – The role of complex carbohydrates in the diet

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0316 (N03001)	Non-starch polysaccharide micronutrient interactions	Glasgow Caledonian University	01/07/97	30/06/00	37,373
AN0317 (N03002)	Cell proliferation and apoptosis in the human colonic mucosa – responses to dietary complex carbohydrates	Newcastle University	01/09/97	31/08/00	116,066
AN0318 (N03003)	Assessment of micronutrient availability in long-term high & low non-starch polysaccharide consumers	Division of Public Health, University of Leeds	01/07/97	31/12/99	24,719
AN0319 (N03004)	DNA replication in colonic mucosa monitored by the BUdR- Comet assay	University of Ulster	01/10/97	30/09/00	83,713
AN0321 (N03005)	Measurement of blood plasma samples as part of non-starch polysaccharide (NSP) study with the University of Leeds	Central Science Laboratory, York	01/04/98	31/03/00	18,200
TOTAL					280,071

AN04 (now N04) – Antioxidants in food

1. Background

Free radicals arise during normal human metabolic processes through the use of oxygen. In addition, environmental pollutants and some drugs can react within the body causing the production of free radicals. Unless quenched, the presence of highly unstable free radicals can result in intracellular and membrane damage. DNA, proteins and polyunsaturated fatty acids of membrane phospholipids, in particular, are targets for attack, disturbing cellular metabolism and eventually impairing cell function. This process is implicated in the pathogenesis of a number of degenerative diseases including cardiovascular disease, some cancers, cataract and connective tissue disorders as well as the ageing process itself.

The epidemiological evidence that the consumption of a diet rich in fruit and vegetables protects against degenerative diseases has been hypothesised to be as a result of the fact that these foods are rich sources of antioxidants, phytochemicals and other nutrients that are known to have antioxidant properties.

However some antioxidant compounds can also be demonstrated to have prooxidant properties at certain levels of intake. Specific antioxidants operate through different mechanisms in the cell. The antioxidant compounds under investigation in this programme are principally vitamins C and E, carotenoids, and non-nutrient compounds such as the flavonoids.

Following the 1996 Review of MAFF R&D it was concluded that future research in this programme should concentrate on human intervention studies *in vivo* to examine the effects of different diets, by using validated biomarkers developed as potential intermediate predictors of disease end points. The focus of this programme continues to be the development of intermediate biomarkers of oxidative stress which could be used to quantitatively study the effects of the antioxidants present in the diet on these biomarkers of potentially adverse effects.

2. Projects

There are currently fourteen ongoing projects in the antioxidant programme. New FSA numbers begin with N04.

The former MAFF project AN0433 has demonstrated that DNA is very readily oxidised on isolation and work-up for analysis. It is difficult to isolate double-stranded DNA from cells. HPLC/ED and GC-MS methods have been used to measure the levels of 8-hydroxy-deoxyguanosine (8OH-dG) levels in pure standards, in synthetic oligonucleotides, and in samples of oxidised DNA. Substantial differences between the two methods were found. The use of NaI method for extracting DNA appeared to produce less artefacts than is the case using pronase digestion. This work has emphasised the importance of measuring oxidised DNA bases by methods which have been shown to give true measures of damage and are consistent and reliable. The inclusion of internal standards in all analyses is essential. These issues are being addressed by a European Standards Committee on Oxidative DNA damage.

N04021, an extension of AN0513 (Optimal Nutrition Programme), has shown that Vitamin C supplements did not show an enhanced level in plasma in individuals with higher than average plasma vitamin C levels. DNA oxidised bases in lymphocytes were measured as a broad measure of oxidative stress. Vitamin C supplements in conjunction with iron did not show increases in levels of mutagenic 8-OH guanine, but there were increases in 5-OH cytosine levels.

N04002 aims to develop a postlabelling assay as a biomonitor of oxidative DNA damage. This type of assay was originally developed to detect radiation induced DNA damage. The assay is being compared with GC-MS and the results are presently being analysed. N04013 is examining the optimisation of vitamin C status using markers of *in vivo* oxidative DNA damage. This project will be looking at the effects in humans of different doses of vitamin C, and then measuring vitamin C levels in cells, markers of DNA damage and of DNA repair at the same time. A supplementation study using a number of different doses is due to start in July 2000.

N04022 is addressing whether the measurement of specific oxidative damage at sensitive, regulatory sites within genes correlates with the results obtained from more generalised methods for the measurement of oxidative damage. This will involve developing the ligation mediated PCR technique and terminal transferase dependent PCR. The genes to be studied are the p53 tumour suppressor gene, where mutations are associated with 50% of human cancers, the pGk1 gene (a housekeeping gene), and k-ras mutations which are associated with lung cancer. Both basal and induced damage by hydrogen peroxide will be measured using HPLC-ED, and/or post-labelling and compared with gene damage measurements made by PCR methods. The first objective will be to develop validated assays for the measurement of *in vitro* oxidative damage in the key genes, to investigate the susceptibility of certain key genes to oxidative damage and subsequently establish systems that can detect modulation of the damage. A human trial is planned using vitamin C if the initial methodological objectives can be achieved.

N04019 is concerned with the modulation of respiratory tract fluid antioxidants through antioxidant supplementation and the impact on individual susceptibility to air pollutants, namely ozone. This study is looking at the antioxidant composition of respiratory tract lining fluid, as well as lung function of healthy volunteers, before and after ozone exposure. The main question of interest is whether or not vitamin C can be targeted to the lung. The next objective will be to use an oral vitamin C dosing ladder, to see whether dietary antioxidant supplementation is effective in modulating the concentration of vitamin C in the alveolar lining fluid, and whether increasing respiratory tract lining fluid ascorbate concentrations will affect other endogenous antioxidants in the same compartment.

N04014 is a stable isotope study examining the metabolism of gamma-tocopherol and its relationship to alpha-tocopherol metabolism. This project initially established the range of vitamin E metabolite (alpha chroman acid; alpha quinone lactone and gamma chroman acid) excretion in normal healthy subjects. It is now known to be feasible to measure three vitamin E metabolites within the same urine sample. In the cohort of normal volunteers, 94% of circulating vitamin E was present as alpha-tocopherol, whereas 6% was present in the gamma form. In contrast, urine from these subjects contained twice as much gamma chroman acid as alpha chroman

acid. This finding suggests that either the metabolism of gamma-tocopherol is considerably faster than alpha-tocopherol or more gamma-tocopherol is directly excreted post ingestion. Deuterated gamma-tocopherol supplementation will be used to examine gamma-tocopherol metabolism in normal individuals.

N04017 is examining the absorption, and pharmacokinetics of urinary excretion of non-nutrient antioxidants such as flavonoids and other phenolics derived from consumption of apple and tomato juices. Although there are over 4000 different flavonoids in the plant kingdom, there are about 50 major flavonoid components in the diet, found in high proportions in fruit (eg apples, pears, grapes, berries of all types and citrus) and moderate to low amounts in vegetables (eg onions, egg plant, green vegetables). This work involves a consortium of three major European laboratories who will determine the bioavailability of flavonoid and phenolic components of fruit juice and in the course of so-doing will validate the analytical procedures through inter-laboratory comparisons. This is the first time that the approach of inter-laboratory validation has been undertaken in the study of flavonoids and the outcome will contain significant analytical information for the research arena of the efficacy of dietary flavonoids and their nutritional value.

N04008 was a study of the bioavailability and efficiency as antioxidants of certain dietary flavonoids. It was shown that following a meal of lightly fried onions, quercetin and isorhamnetin could be detected in plasma, and that concurrent consumption of tomatoes could suppress isorhamnetin uptake. The study also examined the effect of flavonoid supplements on levels of oxidative DNA damage and suggested that high levels of isorhamnetin glycosides could be associated with a decrease in DNA oxidation. Several publications have arisen as a result of this project and a food flavonoid database has been created according to specific criteria allowing the design of low-flavonoid diets for human trials.

N04001 is investigating a new area of interest in the use of F₂ isoprostanes as biomarkers of lipid peroxidation. They are formed during the free radical mediated peroxidation of arachidonic acid independently of the cyclooxygenase pathway. Improved immunoassays for 8-epi-PG_{2α}, along with methods for measuring F₃ and F₄ isoprostanes have been developed. The project has concluded that diet does not appear to contribute significantly to circulating levels of isoprostanes. N04012 is concerned with the validation of five alternative methods of measuring *in vivo* oxidative damage to polyunsaturated lipids. Absolute methods of measuring lipid peroxidation include measurement of F₂ isoprostanes by GC-MS, and malondialdehyde by HPLC. Capillary electrophoresis is being used to examine the effect of peroxides on lipoprotein nature and function. Immunological approaches are being developed using both monoclonal and polyclonal antibodies.

N04006 investigated protein modification in the apo-lipoprotein B100 of circulating LDL in normal individuals. The aims were to investigate whether the amino acid composition in apo-lipoprotein B100 is modified in smokers; whether there is a relationship between vitamin C status and apo-lipoprotein B100 modification in smokers versus non-smokers; and the influence on the tocopherol status of the LDL. The results suggested no difference in the amino acid profile within the apoB100 of the LDL particle for smokers compared to non-smokers. Vitamin C levels and LDL alpha-tocopherol: cholesterol ratios were significantly lower in the female smokers compared to non-smokers, but this difference was not seen in the males.

N04010 is looking at the development and validation of methods for the detailed mapping of DNA damage induced by reactive oxygen species. The objective of the work is to develop biomarkers which capture the 'fingerprint' of DNA sequence hotspots associated with damage by reactive oxidative species.

N04016 also involves looking at developing methods for the measurement of specific oxidative DNA damage. A PCR-based technique for cloning the sites of oxidative damage is being developed base on specific reactions of proteins involved in DNA repair. It has been shown that it is possible to use a system – the his-tagged yOgg1 system – to selectively separate damaged yeast genomic and mitochondrial DNAs from undamaged DNAs.

N04020 is looking at *in vivo* biomarkers that are related to products formed through oxidative damage to proteins. Biomarkers under study include specific protein carbonyls and thiol content, as well as individual amino acid oxidation products, derived from both aromatic and aliphatic residues. Determination of protein oxidation can provide information as to the denaturing radical species, and can inform on both intracellular and extracellular oxidative stress. In addition, the functional effects of oxidative stress can be determined, through for instance studying the effects on ligand receptor binding. The first report on the effects of dietary vitamin C on modulation of plasma protein oxidation *in vivo* has shown a reversible protective effect with a dose of 400mg/day for up to 25 weeks (BBRC 2000: 273, 729-735).

N04015 is an investigation into the effects of dietary vitamins C and E on *in vivo* adhesion molecule expression. In the genesis of atherosclerosis, it is not well understood what factors lead monocytes to bind to an intact endothelium. One of the first stages is the expression of adhesion molecules, and expression of adhesion molecules can be induced by oxidative stress. The purpose of the contract is to study the effects of antioxidants on intercellular communication via adhesion molecules between monocytes and endothelium, specifically looking at transcription factor activation, mRNA transcription and cell surface protein expression. ICAM-1,

E-selectin and VCAM-1 are the focus of interest, as their induction can be mediated by reactive oxygen species and LDL hydroperoxides which signal through the redox sensitive transcription factor NF- κ B. Collaboration with another study, N04011, is involved. This study is looking at the effect of antioxidant supplementation on the atherogenic and thrombogenic potential of monocytes, and in particular of P-selectin-mediated effects. This study is its early stages. Flow cytometric methods have been established and standardised to detect P-selectin expression on platelets, to measure tissue growth factor antigen on monocytes and to measure platelet-monocyte aggregates. Later, the project will involve a dietary supplementation trial of vitamins C and E, to determine whether supplementation can alter a) the expression of P-selectin and tissue factor *in vivo*, b) the production of pro-atherogenic cytokines and chemokines, and c) the regulation of tissue factor expression on monocytes induced by P-selectin mediated cell to cell contact. This trial will also provide samples for project N04015.

N04005 is studying the effects of the antioxidants vitamin C, alpha-tocopherol and lutein on gene expression. The effects of antioxidants on the expression and activity of tissue growth factor and tissue growth factor pathway inhibitor in endothelial cells and macrophages have been studied because of the involvement of these proteins and cells in haemostasis and formation of thrombi.

N04007 is developing biomarkers of free radical damage in blood proteins through the use of specific antibodies. This project aims to produce a series of antibodies, both polyclonal and monoclonal to defined antigens which are known to be formed in oxidatively damaged proteins i.e. 3-nitrotyrosine, di-tyrosine, 4-nitro-phenylalanine, 3-chlorotyrosine and 4-nitro-tryptophan. The benefit of producing antibodies to these antigens is in developing assays which are capable of being undertaken on large numbers of samples at relatively low cost.

A short report was commissioned (AN0462) designed to build on the strengths of this programme by linking the research groups with their counterparts in Europe. An application was submitted to the first call of the Quality of Life sub-programme of FPV to support a concerted action on the 'European Research on the Functional Effects of Dietary Antioxidants (EUROFEDA)'. The application was highly rated and began in January 2000. This will provide improved opportunities for future Europe-wide research collaboration.

3. Dissemination of Results

A number of publications in peer reviewed journals have resulted from work in this programme, and a workshop took place in July 2000 incorporating work from both AN05 and AN04.

4. Comments

The programme adviser was the late Professor Anthony Diplock who has been replaced by Dr. David Lindsay.

AN04 (now N04) – Antioxidants in food

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0423 (N04001)	Isoprostanes: selective markers of peroxidation of different fatty acids in the human body	Kings College, London	01/04/96	31/03/99	26,874
AN0425 (N/A)	Novel antibody based technology for studying the potential ameliorating effects of vitamin E and vitamin C	Aston University	01/04/96	31/03/99	29,110
AN0429 (N/A)	DNA damage and repair; relative responses to antioxidant nutrients in the diet	IFR, Institute of Food Research	01/07/96	30/06/99	27,935
AN0432 (N04002)	Development of a postlabelling assay as a biomonitor of oxidative DNA damage in humans	Leicester University	01/10/97	31/05/00	60,068
AN0434 (N04003)	Biomarkers for <i>in vivo</i> oxidative damage to DNA: a validation study	Kings College, London	01/04/97	31/03/99	27,136
AN0437 (N/A)	Antioxidants and the expression of genes in cells: the maintenance of health in vascular tissues	Royal Free Hospital School of Medicine	01/04/97	31/03/00	88,268
AN0438 (N/A)	Antioxidant nutrients and the cellular adaptation to oxidative stress in man	Liverpool University	01/06/97	31/05/99	25,501
AN0439 (N04006)	Early markers of oxidative stress <i>in vivo</i> through identification of protein damage in circulating low density lipoproteins	Guy's, Kings and St. Thomas' School of Biomedical Science	01/01/98	31/12/99	51,351
AN0440 (N04007)	Biomarkers to free radical damage to blood proteins by peroxynitrite and hypochlorous acid	Royal Free Hospital School of Medicine	06/10/97	05/10/00	71,591

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0445 (N04008)	Dietary flavonoids and carotenoids; bioavailability and efficiency as antioxidants (formerly AN0518)	Rowett Research Institute	01/12/95	28/02/99	15,000
AN0450 (N04010)	Development & validation of a methodology for the detailed mapping of DNA damage induced by reactive oxygen species	University of Swansea	01/05/98	30/04/01	58,138
AN0451 (N04011)	The effect of antioxidant supplementation on P-selectin mediated monocyte atherogenic and thrombogenic potential	Leicester University	01/10/98	30/09/01	104,393
AN0452 (N04012)	A consensus approach for validation of 5 alternative methods of measuring <i>in vivo</i> oxidative damage to polyunsaturated lipids	Leicester University	01/10/98	31/12/01	86,146
AN0453 (N04013)	Optimisation of antioxidant nutrient vitamin C using markers of <i>in vivo</i> oxidative DNA damage	Leicester University	01/04/98	31/03/01	86,455
AN0454 (N04014)	Gamma-tocopherol: A stable isotope study examining its metabolism and relationship to alpha-tocopherol	St Thomas' Hospital	01/07/98	30/06/01	111,216
AN0455 (N04015)	An investigation into the effects of dietary vitamin C and E on <i>in vivo</i> adhesion molecule expression	Aston University	01/10/98	30/09/01	67,406
AN0456 (N04016)	PCR based technique for cloning sites of oxidative DNA damage & measuring <i>in vivo</i> protective effects of dietary antioxidants	Liverpool University	01/04/98	31/05/01	46,769
AN0457 (N04017)	Absorption & excretion of flavonoids and phenolics in humans after beverage consumption & validation of analytical procedure	United Medical & Dental School Free Radical Research	01/10/98	31/01/01	70,130

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0458 (N04018)	Absorption & excretion of flavonoids and phenolics in humans after beverage consumption & validation of analytical procedure	Glasgow University	01/10/98	31/01/01	60,772
AN0459 (N04019)	Modulation of respiratory tract lining fluid antioxidants through antioxidant supplements: impact on individual susceptibility to air pollution	St Thomas' Hospital, Rayne Institute	01/09/98	31/08/01	92,234
AN0460 (N04020)	Development & validation of <i>in vivo</i> biomarkers of oxidative damage to proteins	Aston University	01/07/98	30/06/00	63,959
AN0461 (N04021)	Potential problems of vitamin C supplementation – a proposed resolution of an imminent problem? (extension to AN0513)	Guy's, Kings and St. Thomas' School of Biomedical Science	01/09/98	30/11/99	68,517
AN0462 (N/A)	Facilitation & co-ordination of research relevant to the objectives of MAFF anti-oxidant programme within the EU	Euro Science Perspectives Ltd	01/02/99	31/07/99	18,687
AN0463 (N04022)	Does the measurement of specific oxidative damage at sensitive sites correlate to broad biomarkers & risk of disease?	Leicester University	01/10/99	31/10/02	22,080
TOTAL					1,379,736

AN05 (now N05) – Optimal nutrition status

1. Background

The concept of Optimal Nutrition Status originally centred around the prevention of nutritional deficiencies, but now it has widened considerably to include the maintenance of optimal health. This is the result of an increasing awareness of the importance of micronutrients and biologically active components in the diet in reducing morbidity and increasing longevity with a better quality of life. For example, these components are known to play a role in the maintenance of heart health, colon health and bone health, and in the protection against diabetes, anaemia and impaired immune function.

Policy objectives

There are a number of policy drivers for the Optimal Nutrition Status programme. The first major policy driver was the 1991 report from the Committee on Medical Aspects of Food and Nutrition Policy (COMA), in which the Committee provided an assessment of the science available at that time on the links between a range of micronutrients and the maintenance of good health. COMA acknowledged that, although in some cases the evidence on which dietary requirements are based is reliable experimental data, in others the evidence was much scarcer.

Other policy drivers include the research recommendations of subsequent reports from COMA, which have addressed the links between diet and cardiovascular disease, cancer and bone health. More recently, *Our Healthier Nation* (1999) provides an overarching view of the role of diet and its interactions with other factors which determine health in four key areas – cardiovascular disease, cancer, accidents and mental health.

The major policy objective for this programme continues to be the need to conduct appropriate research in those areas of highest relevance to public health where extant scientific evidence is poorest, thereby providing the scientific basis for population-level guidelines as to the optimal level of intake of micronutrients from the UK diet.

Scientific and technical objectives of the Optimal Nutrition Status research programme

The 1996 Review of MAFF Nutrition R&D recognised the direct policy relevance of this programme to the refinement of dietary reference values, but thought that the then primary policy driver of the 1991 COMA report had led to a fragmented approach in its concentration on those nutrients for which specific gaps in knowledge had been identified. The scientific and technical objectives of this programme have therefore been refined. The programme now aims to use research means to address two specific kinds of issue.

First there is a need to understand the links between optimal nutrition status and the maintenance of good health. Research will be justified where there is an explicit, established link between functional markers of nutrient status and a health outcome of relevance to public health priorities. This is a key area for interaction and co-ordination with other public sector research funders whose remits cover molecular mechanisms in disease progression and prevention.

Given the overt link to the maintenance of good health, it is also a priority to understand the potential interactions between micronutrients at different stages of life in the reduction of risk of specific disease, for example interactions between calcium, vitamin D and vitamin K in the area of bone health.

Second, there is a need to develop accurate measures of the bioavailability of nutrients in food. Bioavailability has classically been determined by *in vitro* techniques simulating digestion and passive diffusion across the gut wall, or by *in vivo* studies using laboratory animals. However, these methods take account of neither the myriad interactions that may occur between nutrients and other components of food to influence their uptake from the gut in humans, nor the effective transport of the nutrients from the site of absorption to their target tissues in humans. These factors are key to understanding the relationships between dietary nutrients and the physiological processes that maintain good health.

Three broad research objectives arise from this consideration of the processes that link ingestion of nutrients in food to changes in tissue function that are relevant to health:

- Measuring the fraction of ingested nutrients used to meet functional demands in target tissues
- Developing, for each micronutrient or group of nutrients, functional markers of status – these are physiological or biochemical factors that relate to target tissue function and are sensitive to changes in intakes or stores of those nutrients;
- Using human intervention studies to determine the dose-response relationship of micronutrients against tissue function, and the nature and extent of inter-individual variations in response, to identify optimal status – the micronutrient intakes at which functional markers reach an optimal value in whole populations.

2. Outputs

20 strategic research projects have been funded in this programme during FY 99/00. Seven new strategic research projects started in 1997 and are due to finish between 1998 and 2000. Nine projects started in 1998 and are due to finish between 1999 and 2001. Two projects (see below) started and finished in FY99/00. Five new projects started in FY99/00 and are due to finish by 2003. The budget for this whole programme was £1.6 million for FY 99/00.

During FY99/00, the co-incidence of EU and MAFF timetables for funding meant that projects which had been successful in receiving EU funding under the Framework 5 Quality of Life Programme could apply for 'top-up funds' from MAFF/FSA. UK Contractors in two EU projects, FolateFunctHealth and Osteodiet have been funded under this arrangement (new FSA project codes N05024 and N05025 respectively).

Important outputs from the programme which have emerged this year have included the following issues:

Project AN0554 was commissioned to put the findings from this programme in a wider scientific context. Therefore the project aimed to assess the AN05 research programme using the combined techniques of questionnaires, appraisal of international databases, informal meetings with current contractors and discussions of draft reports at workshops which were held in late 1999. The conclusion of this review, presented to FSA in early 2000, stated that 'good progress is generally being made, the focus of the programme objectives would appear to be appropriate to the questions that need to be addressed, and the projects funded in all but the early stages of the programme are consistent with the objectives'. However the broad range of nutrients associated with this programme has inevitably meant that funding and attention any particular nutrient attracts is relatively small, which inevitably hampers overall progress. A short summary of the review process will be published in the British Nutrition Foundation Bulletin and further articles are planned to give more detailed discussion of the scientific content of the document.

Project AN0536 investigated the bioavailability of vitamin E in normal volunteers when the energy content of the meal provided by fat was 30, 35, 40 or 45%. No significant difference in vitamin E uptake was observed, indicating that the fat content of a meal *per se* does not influence vitamin E bioavailability. The contractors therefore conclude that the fat content, or indeed the vitamin E content of a meal does not dictate the amount of vitamin E absorbed into the body. Compensation mechanisms are available in normal individuals which can maintain adequate vitamin E uptake in these circumstances. Together these findings provide reassurance that current recommendations regarding decreased fat intake should have little or no impact on vitamin E intake, at least in normal, healthy individuals.

Project AN0555 was a short project funded during FY99/00 which was a follow up project to AN0504 which established a provisional database for vitamin K and arose out of the needs of two ongoing projects which are investigating the effect of vitamin K and other micronutrient on bone health- AN0525 and AN0532. The objective was to update the provisional vitamin K1 food database so that foods relevant to all sectors of the UK, by age and ethnicity, are fully represented. This will also ensure that K1 values on a substantial number of foods can be reviewed for inclusion in UK food composition tables.

The latest COMA report from the Department of Health was published during FY99/00. This report on *Folic acid and the prevention of disease* focussed almost exclusively on the ability of folic acid to reduce the incidence of neural tube defects, and issues and strategies relating to folate fortification of food. Several projects within AN05 address the issue of the bioavailability of folate from different sources. AN0538 finished during FY99/00 and concluded that the relative absorption of folic acid was 49% from white bread, and 31% from breakfast cereal, compared to folic acid capsules. This indicated that the matrix of these cereal-foods has an inhibitory effect on intestinal absorption.

Projects AN0546 and AN0550 are performing further investigations into the bioavailability of different forms of folate. The COMA Report mentioned the possible role of folic acid in reducing homocysteine and the possible implications of this in the prevention of cardiovascular disease. Projects AN0526 and AN0534 on an intervention study to address this issue are due to finish in FY00/01.

3. Dissemination Activity

The results of work supported within this programme have been published by individual contractors in the scientific literature and a number of other papers are at various stages of preparation and submission to journals.

The recent review of the whole AN05 programme will be published as detailed above. A report of the 2000 Annual meeting will also be published to summarise some of the achievements of Programmes AN05 and AN04 in the science of antioxidants.

4. Future Activity

The programme adviser has visited all projects at least once during FY 1999/00 and is now planning further visits during FY 2000/1.

The annual meeting took place in July 2000. It focussed on the projects concerned with the bioavailability of the antioxidant nutrients. Key contractors from the Antioxidants in Food research programme reviewed their work on the mechanism of oxidation of lipids, proteins and DNA.

5. Comments

Co-operation between the Programme Advisers responsible for MAFF's Nutrition Research Programmes has continued this year and has, again, been mutually beneficial.

The programme adviser is Dr Margaret Ashwell OBE.

AN05 (now N05) – Optimal nutrition status

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0525 (N05001)	Evaluation of the effects of a 2-year intervention with calcium & vitamins D and K on bone health in elderly women	Dundee University	01/05/97	30/04/00	151,641
AN0526 (N05002)	Dietary folate, homocysteine and endothelial function: A study of the interaction with methylene-tetrahydrofolate	University College of Wales	01/05/97	30/04/00	100,521
AN0528 (N05003)	Bioavailability, pool size and turnover of vitamin C using stable isotopically-labelled vitamin	Medical Research Council	01/10/97	31/03/01	36,125
AN0532 (N05004)	Dietary factors protecting against osteoporosis in the seventh and eighth decades of life	Institute of Public Health, Cambridge	01/07/97	30/06/00	42,880
AN0533 (N05005)	Model systems, <i>in vitro</i> and <i>in vivo</i> , for predicting the bioavailability of lipid soluble components of food	IFR, Institute of Food Research	01/11/97	30/04/01	0
AN0534 (N05006)	Dietary folate, homocysteine and endothelial function: the potential role of oxidative stress in vascular damage	University of Sheffield	01/10/97	30/09/00	53,565
AN0536 (N05008)	The use of deuterated tocopherol to study vitamin E metabolism in normal subjects ... (formerly AN0428)	United Medical and Dental School – Free Radical Research	01/04/96	31/08/99	0
AN0537 (N05009)	The use of deuterated tocopherol to study vitamin E/vitamin C/glutathione interrelations in human ... (formerly AN0436)	St Thomas' Hospital, Rayne Institute	01/04/97	31/05/00	78,952
AN0543 (N05010)	Biochemical and molecular markers of functional selenium status in man	Rowett Research Institute	01/04/98	31/03/01	110,708

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0544 (N05011)	Speciation, bioavailability, biopotency and functional markers for D vitamers	Laboratory of the Government Chemist	01/06/98	31/03/99	12,169
AN0545 (N05012)	Functional markers of selenium in man	University of Liverpool	01/04/98	31/03/01	87,818
AN0546 (N05013)	Bioavailability of folic acid and natural folates: studies using the functional marker plasma homocysteine	University of Ulster	01/06/98	30/11/01	169,797
AN0548 (N05014)	The bioavailability of iron, zinc and copper in meat- containing and vegetarian diets in the UK	Central Science Laboratory, York	01/10/98	31/12/01	49,520
AN0549 (N05015)	The bioavailability of iron, zinc and copper in meat- containing and vegetarian diets in the UK	IFR, Institute of Food Research	01/01/99	31/12/01	194,965
AN0550 (N05016)	Is folic acid considerably more effective than folates in raising folate status?	IFR, Institute of Food Research	01/07/98	31/08/01	91,977
AN0551 (N05017)	Test of predict model for bioavailability Fe (Zn & Cu) from mixed meals using simple <i>in vivo</i> human test for low doses of Fe	Kings College, London	01/01/99	31/12/01	80,226
AN0552 (N05018)	The importance of meat consumption in regulating vitamin D metabolism and promoting bone health	Manchester Royal Infirmary	01/10/98	30/09/01	149,841
AN0553 (N05019)	The development of functional markers of optimal nutritional status for copper	University of Ulster	01/10/98	31/12/01	84,321
AN0554 (N05020)	Critical overview of research in the field of optimal nutrition status	British Nutrition Foundation	01/04/99	31/12/99	78,008
AN0555 (N05021)	Extension and verification of the provisional UK phylloquinone (k1) food composition database	Dundee University	01/09/99	30/04/00	12,489
TOTAL					1,585,523

AN06 (now Q01) – Food authenticity

1. Background

Selling food which is not as it is described misleads, and can defraud consumers. Misdescription can take many forms, from the undeclared addition of water or cheaper materials (adulteration) to the wrong quantitative declaration of ingredients, or the incorrect declaration of origin (species or geographical). The main rationale of this programme is the development of analytical methods, which enable Government, enforcement authorities and industry to ensure that products are correctly described and labelled, and that consumers are adequately protected.

The objectives for the research in this programme are as follows:-

- To identify and measure specific biochemical or chemical markers, or use physical parameters and behaviour, for establishing the authenticity of food;
- To develop an understanding of the variability of these markers or parameters due to natural variation, climate, origin, species and other factors including processing, and, if necessary, develop and extend databases to embrace this variability;
- To develop and refine methods for identifying foods or ingredients in compound-foods so that they can be measured quantitatively;
- To encourage the development of simpler or more rapid methods of testing food authenticity;
- To establish the accuracy, precision and suitability, of methods developed above through rigorous evaluation or collaborative testing; for Food Standards Agency surveys or for enforcement and industry.

The first phase of the programme commenced in 1993 and was reviewed in July 1998. The second phase started in 1998 and is expected to run until 2003, although the direction of the programme may be amended to take into account the values and objectives of the Food Standards Agency. The total spend in the financial year 1999/2000 was ca. £1m.

2. Outputs

The programme continues to investigate a wide range of novel approaches applied to authenticity problems in different foods. Although spectroscopic techniques continue to provide the strong analysis of markers for

geographical origin of plant components, the increasing number of projects using DNA technology is indicative of the success of using genetic markers to resolve authenticity problems. In particular five out of the eleven completed projects in 1999/2000 were based on DNA techniques.

Geographical Origin

Novel approaches to determine geographical origin have been examined in four projects. AN0676 investigated the application of isotopic ratios and trace element analysis to differentiate between rice from different geographical origins using multivariate analysis. Since rice varieties are to a large extent linked to geographical origin, a new DNA method to identify the variety of a rice based on simple sequence repeat polymorphism (SSRP), was also developed. This technique can be used quantitatively by applying fluorescent tagged primers. After development, all the approaches were used to examine commercial samples of Basmati rice by comparing their results to a database of authentic varieties of rice. Although combined isotopic and trace element analysis can distinguish country of origin, the technique is not able to distinguish mixtures of Basmati varieties and long grain rice from the same origin. On the other hand, SSRP applied to rice is able to measure reasonably accurately admixtures of varieties. Further work on developing this interesting DNA technique is being undertaken.

Trace element analysis was also used in conjunction with analysis of the oligosaccharides and volatile compounds as a means of characterising the floral origin of honey (AN0684). Work was carried out on 48 samples of nine floral varieties. Trace element analysis showed successful separation by floral type, reliability was increased where higher sample numbers of a particular floral type were available. This was also true for GC analysis of the oligosaccharides. However, the data was too widely spread for the GC-MS analysis of volatiles to give conclusive discrimination between floral types.

Two different approaches have been used to verify the geographical origin of wines. AN0683 examined natural yeast populations isolated from grapes, must and wine, which have been shown to be genetically distinct between geographical regions and between strains of the same species within oenological "terroirs". Further work is examining the potential use of DNA fingerprinting techniques to identify the geographical origin of yeast in wine. AN0678, on the other hand, used stable isotope measurement of wine proteins and volatile substances as an indicator of geographical origin. No single parameter was able to discriminate between countries or regions of origin. However, statistical analyses using combinations of isotopic parameters (including wine alcohol) were more successful in discriminating between wines from different regions.

Verification of Wine Vintage

Radio-carbon dating is the standard method used for verifying vintages of alcoholic drinks, but, as carbon-14 background levels decline following the cessation of nuclear testing, scintillation counting is no longer sufficiently precise for this measurement. AN0688 demonstrated that accelerator mass spectrometry can provide precise isotope measurement of whisky and wine with the potential for vintage determination for products of a single, known geographical origin (e.g. chateau-bottled wines).

Authenticity of Fruit Juices and Pulps

Projects AN0672 and AN0691 have further developed molecular assays for the identification of fruit pulps and fruit juices. A method to detect grapefruit juice in processed orange juice has been developed and validated. Also assays have been developed for the identification of different fruit pulps in processed food products. These assays have been evaluated and are capable of identifying fruit species present in some processed products such as yoghurts, but not in jams.

Species Origin

Two projects to determine species or anatomical origin of meat components have been successfully completed. AN0695 was designed to develop a rapid and easy DNA method to detect small quantities of chicken or turkey muscle in cooked meat products. The PCR primers used, produced amplicons in mixtures of chicken and turkey, and *vice versa*, without showing any signs of cross-reactivity with other commercially used meat species. Vistra Green was used to detect the presence of amplicons and was found to be sensitive, rapid and easy to use. For the method to be effective on highly processed products where the DNA is highly degraded, it would be necessary to redesign the primers to produce a shorter amplicon of the order of 100-150bp.

The overall aim of project AN0699 was to develop an assay to identify neuronal tissue from cows, sheep and goats, and to enable the identification of bone marrow from chicken as a marker for the presence of mechanically recovered chicken meat. Both approaches used methylation sites to determine whether the genes producing these components were switched on or off. It was not possible to find a gene to give robust differences in methylation events for bone marrow synthesis. However, the assay was successful for distinguishing neuronal tissue from muscle of cow or sheep origin even in cooked mixtures. Further work is required to fully evaluate and validate the initial findings and to develop the technique as a commercial assay.

Authenticity of Dairy Products

A feasibility study (AN06103) has been completed to assess a range of analytical methods and compositional parameters for detection of protein standardisation of drinking milk from cows. The study recognised, at the outset, that it was unlikely that any one single parameter would show whether milk had been protein standardised. All data was therefore subject to multivariate statistics to choose which of the parameters might characterise standardised milks. The research has produced a comprehensive database on the nature and chemical composition of a range of protein standardised and non-standardised milks samples. This has good potential for application to a larger geographic survey of UK milk samples that may be undertaken in the future.

Research (AN0694) evaluating a suite of analytical methods to distinguish cheese analogues (i.e. imitation cheeses) from genuine cheese in foods has been completed. In order to test whether a cheese is genuine, an imitation or a mixture of genuine with imitation cheese, a decision tree approach, which compares data from the unknown sample with ranges found for genuine cheese, has been successfully developed.

Vegetable Fats in Chocolate

The improvement of methodology to determine the amount of vegetable fats in chocolate was an important issue during the negotiation of the revised EC Chocolate Directive. The accuracy of the triglyceride determination is greatly improved if the identity of the vegetable fat is known. AN0673 used multivariate analysis of sterol breakdown products and triglyceride analysis to characterise and identify blends of vegetable fats. Whereas it was not possible to positively identify unknown blends of fats in all cases, the technique is very useful to confirm that samples taken during in-factory inspections are in fact those used in the manufacture of the chocolate.

3. Dissemination Activity

In recognition of the increasing number of projects within the programme that utilise DNA techniques, a DNA review workshop was held in July 1999. This allowed contractors to present the results of projects to their peers and discuss their work. The workshop concluded that a wide range of DNA techniques could be used to successfully solve various authenticity problems. It also identified common problems and suggested ways in which they may be overcome and this has prompted the commissioning of further projects to address these issues. The publication of the results of research projects in relevant journals is encouraged.

4. Future Activity

The 1998 programme review had broadly endorsed the objectives and priorities of the programme, which in future will have to meet any new priorities of the Food Standards Agency. The importance of method evaluation was recognised and will need to be further implemented in the future work of the programme. The importance and uncertainties of DNA techniques have also been highlighted and are being addressed in some newly commissioned projects. The wider public divulgence of DNA techniques developed in the programme will be given further consideration. European co-operation is also important in authenticity research, and encouragement of contractors to find European collaborators will continue as will the investigation of other avenues of co-operation.

5. Comments

The Programme Adviser is Dr Mark Woolfe, Food Labelling, Standards and Consumer Protection Division.

AN06 (now Q01) – Food authenticity

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0673 (Q01001)	Improved methodology for the identification of non-cocoa fats in chocolate	Central Science Laboratory, York	01/04/97	30/04/99	4,775
AN0676 (Q01038)	The development of isotopic analysis and DNA polymorphic markers to determine the geographical & cultivar origin of premium long grain rice	Central Science Laboratory, York	01/05/97	30/04/99	14,800
AN0676 (N/A)	The development of isotopic analysis and DNA polymorphic markers to determine the geographical & cultivar origin of premium long grain rice	Nottingham University	01/05/97	30/04/99	16,661
AN0680 (N/A)	Development & validation of immunochemical methods for the detection of MRM in meat products	Nottingham Trent University	01/04/97	31/03/99	10,030

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0683 (N/A)	Exploitation of the genetic variability in natural yeast populations to determine the geographical origin of wine	National Institute of Agricultural Botany	06/01/98	31/03/99	11,434
AN0685 (Q01002)	Detection of meat species in fresh and processed food: Production & use of mabs reactive with insoluble muscle protein desmin	Nottingham Trent University	28/09/98	27/09/00	38,661
AN0686 (Q01003)	Differentiation of species of meat in particular cooked products by DNA methods	Laboratory of the Government Chemist	01/04/98	30/09/99	55,112
AN0687 (Q01004)	Species identification of raw and heat processed fish from computer data bases of electrophoretic protein profiles	Rowett Research Institute	01/04/98	31/03/00	27,299
AN0689 (Q01005)	Speciation of processed meat and fish products based on the actin multigene family	Nottingham University	01/08/98	31/07/00	59,394
AN0690 (Q01006)	Identification of species in processed/composite fish/ seafood products using DNA-based techniques	Rowett Research Institute	01/04/98	31/03/01	35,388
AN0691 (N/A)	A molecular approach for the identification of fruit pulps in food products	Leatherhead Food Research Association	01/04/98	31/03/99	7,386
AN0692 (Q01007)	The development of a rapid and cost efficient assay for non- permitted cellulases in fruit juice	IFR, Institute of Food Research	01/04/98	31/03/00	36,983
AN0693 (N/A)	Development of a methodology for the identification of bottled waters that have been treated with ozone	Water Research Centre	01/04/98	31/03/99	10,583
AN0694 (Q01039)	Evaluation of a suite of methods to distinguish cheese analogues from genuine cheese	Laboratory of the Government Chemist	01/04/98	31/03/99	2,000

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0696 (Q01008)	Detection of offal in cooked meats by protein cleavage & visualisation of tissue specific peptides by electrophoresis	Central Science Laboratory, York	01/07/98	30/06/00	44,300
AN0697 (Q01009)	Evaluation of dielectric spectroscopy in the measurement of added water & in detection of offals & MRM in meat products	Leatherhead Food Research Association	01/08/98	31/07/01	43,447
AN0698 (Q01010)	Quantitation of meat species in meat & meat products using TaqMan PCR	Campden & Chorleywood Food Research Association	01/09/98	31/08/00	61,300
AN0699 (Q01011)	Optimisation & validation of DNA assay for detect of select non-muscle tissues in meat prods using tissue specific DNA modification	Laboratory of the Government Chemist	01/11/98	30/11/99	61,054
AN06100 (Q01012)	The development of rapid & novel Nuclear Magnetic Resonance (NMR) techniques for measuring water in meat	Central Science Laboratory, York	01/08/98	31/07/99	11,079
AN06101 (Q01013)	Varietal identification of potatoes by DNA profiling	National Institute of Agricultural Botany	01/09/98	31/08/00	45,492
AN06102 (Q01014)	The development of methods that can detect protein standardisation of cows' drinking milk	Central Science Laboratory, York	01/09/98	31/08/00	31,542
AN06103 (Q01040)	Development of methods to detect protein standardisation of cows' drinking milk	Laboratory of the Government Chemist	01/09/98	30/11/99	24,790
AN06104 (Q01015)	Develop methods to detect hazelnut oil in olive oil by analysis of volatiles and polar components	Reading University	01/05/99	31/05/00	30,418
AN06105 (Q01016)	Fluorescent molecular beacons for quantitative detection of species-specific markers in DNA isolated from food mixtures	Nottingham University	01/06/99	31/05/00	25,730

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN06106 (Q01017)	Detection of hazelnut oil addition to olive oil	Central Science Laboratory, York	01/06/99	30/04/00	38,181
AN06107 (Q01018)	Develop & validate methods to determine the origin of milk butter & cheese	Central Science Laboratory, York	01/04/99	31/03/02	36,500
AN06108 (Q01019)	Apply GC-Pyrolysis-IRMS to determine the authenticity of vegetable oils, wine, fruit juices and flavours	Central Science Laboratory, York	01/07/99	31/03/01	31,600
AN06110 (Q01020)	Exploit the genetic variability in natural yeast populations to determine the authenticity of certain wines	National Institute of Agricultural Botany	01/04/99	31/03/01	36,167
AN06111 (Q01021)	Within species discrimination of meat cuts evaluate a novel approach using DNA phage display & protein technologies	Rowett Research Institute	01/07/99	30/06/02	22,098
AN06113 (Q01041)	Methods for the detection of rectified grape and apple juice in fruit juices	Laboratory of the Government Chemist	01/04/99	30/09/99	36,090
AN06116 (Q01024)	Nitrogen factors for turkey meat	RSC Analytical Methods Committee	01/10/99	30/09/00	3,000
AN06117 (Q01025)	Development of a method for the detection of adulteration of basmati rice	Nottingham University	01/06/99	30/11/99	34,500
AN06118 (Q01026)	Further development and validation of assays for fruit authentication (ext. to AN0672 & AN0691)	Leatherhead Food Research Association	01/06/99	30/04/00	36,000
AN06119 (Q01027)	Adulteration of olive oil with hazelnut oil: to enable detection of hazelnut oil in virgin & refined olive oils	Marine Laboratory – Aberdeen	01/10/99	30/09/00	15,150
AN06120 (Q01028)	Determination of the authenticity of virgin olive oil by DNA fingerprinting of yeast	National Institute of Agricultural Botany	01/12/99	31/03/01	18,075
TOTAL					1,017,019

AN08 (now N08) – Dietary surveys and nutrients in food

1. Background

Accurate and up-to-date information on the amounts of food eaten in the UK and its nutritional value are important for a number of reasons. Government policy requires a robust scientific basis for determining links between diet and disease, for providing sound advice on diet and health to the public and for monitoring progress towards nutrition-based public health targets.

Government spend on surveys of diet, nutrition and nutrients in food is substantial, exceeding, on average, £1 million per year. The main objective of this research programme is to ensure that these surveys are cost effective and that the data they generate are robust, of high quality, and are able to be translated effectively into information to support policy development.

Research conducted in this programme, which was established in 1991, falls under three main areas:

- the development of improved methods of nutrient analysis, particularly the separation and measurement of the different forms of certain nutrients that occur in foods;
- the validation of current methods and development of improved methods for collecting reliable quantitative data on habitual food consumption;
- appropriate further analysis of data from Government dietary surveys to investigate factors associated with nutrient intake.

The cost of the programme in 1999/2000 was *ca.* £315k.

2. Outputs

Achievements of the programme to date

This programme has made a significant contribution to advancing knowledge on methodologies for both nutrient analysis and dietary surveys. Additionally, it has provided added value to Government dietary surveys (notably the joint FSA/DH National Diet and Nutrition Survey programme and MAFF's National Food Survey) by further interrogating the extensive datasets produced from the surveys. (The Food Standards Agency advises on nutritional aspects of MAFF's National Food Survey.)

Two particularly notable achievements are:

- The publication of *Food Portion Sizes: a photographic atlas*, which was developed under this programme, and which has quickly become established as invaluable dietary assessment tool for researchers.
- A statistical modelling approach which provides estimates of average nutrient intake by types of person, using household purchase data from the National Food Survey. This has attracted much interest and was the subject of a special feature in the 1998 National Food Survey annual report, published in 1999.

Outputs for 1999/2000

Further analysis of data from the National Diet and Nutrition Surveys (NDNS)

Following the successful completion of projects investigating factors affecting the micronutrient status of older people in Britain, as reported in the 1997-1998 Annual Report, two projects analysing data from the NDNS of children aged 1½-4½ years were commissioned in 1998. The first project was described in the 1998-1999 Annual Report.

The second of these projects is now complete. It concluded that 6% of children had inadequate intakes (defined as below the lower reference nutrient intake) of vitamin A; 13% had inadequate intakes of iron, and 16% had inadequate intakes of zinc. A total of 2% of children had inadequate intakes of all three micronutrients. Low intakes of these micronutrients were consistently associated with lower socio-economic status. The authors suggest that adequate consumption of cereals, milk, meat, eggs, fruit and vegetables should enhance intakes and status. However, they advise that overdependence on milk, through its substitution for iron-rich or iron-enhancing foods, may put younger children at risk of poor iron status. This risk disappears when moderate-to-high amounts of these foods (e.g. meat and/or fruit) are consumed concomitantly or with suitable separation from high-calcium meals.

3. Dissemination Activity

Results of several of the projects in this area of the programme have been discussed at a wide range of scientific meetings and published in the scientific literature.

4. Future Activity

The programme will continue to explore methods for improving the assessment of diets in households and individuals, and their interpretation. New projects commencing in 2000 will seek to:

- identify dietary methodology for those on a low-income;
- review methodology for estimating non-milk extrinsic sugars;
- review the NDNS of young people aged 4 to 18 years to examine intakes, status and other determinants of vitamin A, iron, zinc and copper;
- consider the implications for dietary survey methodology of eating habits, food preparation and serving practices in ethnic populations.

Future priorities will again be kept under review with the help of advice from a number of sources including the Food Standards Agency's Working Parties on Dietary Surveys and on Nutrients in Foods. A review of the programme to date is planned.

5. Comments

The Programme Manager is Mrs Susan Church.

AN08 (now N08) – Dietary surveys and nutrients in food

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0835 (N08001)	Detecting and modelling mis-reporting of food intake with special reference to underreporting in the obese	Rowett Research Institute	01/10/97	31/03/01	96,384
AN0844 (N08005)	A review of the NDNS survey of children aged 1.5 to 4.5 years to examine intakes & nutritional status of Fe, Zn and Vitamin A	Dunn Clinical Nutrition Centre	01/07/98	30/06/99	19,358
AN0845 (N08006)	Total diet study	British Market Research Bureau Ltd	01/01/98	31/12/02	19,430

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0846 (N08007)	Food wastage pilot survey	Office for National Statistics	04/05/98	28/02/99	7,200
AN0847 (N08008)	Total diet study	IFR, Institute of Food Research	01/04/98	31/12/02	61,932
AN0848 (N08009)	The effects of micronutrient interactions on iron status using the NDNS survey of children	Southampton University	01/04/98	31/03/99	12,538
AN0850 (N08011)	Systematic review of food frequency questionnaires	University of Leeds	01/05/99	14/04/00	44,631
AN0851 (N08012)	Develop & validate a routine HPLC method for the determination of folates & folic acid in foods	Laboratory of the Government Chemist	01/04/99	30/10/00	54,096
TOTAL					315,569

AN09 (now N09) – Food acceptability and choice

1. Background

Diet has a significant influence on health, and it is therefore important to understand what motivates consumers in their choice of foods. The Food Standards Agency's Nutrition Division, which is the budget holder for this programme, is involved in developing policy, based on sound science, which will encourage the adoption of healthy, balanced dietary choices. A particularly important aspect of this is the provision of information to enable health professionals and others to formulate practical strategies to bring about dietary change. The *Food Acceptability and Choice* research programme was established in order to provide a scientific basis to facilitate these aims.

The programme commenced in April 1991. In all, 27 projects have been commissioned, of which 3 will not begin until 2000/2001 and 19 have finished, including two during the year under review. One project started in 1999/2000. One project has been extended and three new projects will start in 2000/2001.

The total spend for this programme in 1999/2000 was ca. £477,000

2. Outputs

Among the projects completed during the current period are a written review of the programme, intended to summarise its major findings to date, its strengths and weaknesses and the gaps in knowledge still to be explored. The written review was intended as a basis for a major programme review, which took place during the year (see paragraph 4, below). The other completed project was an exploratory development of a healthy eating quiz, intended to enable a member of the public to identify the food choices which can categorize their own diet in terms of fat content, and to alter those choices if necessary.

3. Dissemination Activity

Key results from final reports are outlined in short articles in the Food Standards Agency Food Safety Information Bulletin on a regular basis. Until the Agency moves to its own building the final project reports continue to be held by MAFF library and copies can be obtained by contacting the librarian. Contractors to this programme have had a good record of writing scientific papers and many arising from earlier projects have been published. The written review of the programme has recommended a number of ways of increasing awareness of programme results among dieticians, health professionals and opinion formers. A food journalist, who was among the team working on the written review, produced a series of draft summary sheets of most of the completed projects, written in layperson's terms. It is hoped that these will be placed on the Agency website in 2000.

4. Major Programme Review

A major review of the programme which included all those projects not covered by the mid-term review of 1993, took place in June 1999 over two days at an external venue. The aim of the review was to consider how well the projects have met the scientific and policy objectives of the programme, assess the projects' scientific merit and value for money and make recommendations about the future direction of the programme. Some of the lead researchers from completed and on-going projects were invited to give presentations in front of a small audience of interested parties including a panel of external independent experts in the field of nutrition and health who acted as reviewers. In addition, the external reviewers conducted sessions at which the projects and the programme as a whole were assessed and discussed. The reviewers, both written and external, commended the far sighted and innovative nature of the programme and recommended that it continue, with an emphasis on dietary interventions resulting in outcomes of a practical nature and on more active dissemination of the results. These recommendations, which are already being addressed, are intended to become a major feature of the programme in future years.

5. Future Activity

In 2000/01, a number of primary school-based projects to encourage increased fruit and vegetable consumption, and to develop a strategy to prevent childhood obesity, will continue.

Other on-going projects include further work in Northern Ireland on the barriers to development and uptake of reduced fat foods, and an extended family-based project in the north-east of England designed to encourage consumption of starchy foods and test the acceptability of increased intake.

New projects due to begin in 2000/01 include: the development and evaluation of an interactive CD-ROM to encourage healthy eating for secondary school children; determining the extent to which an intervention designed to teach cooking skills to those on a low income improves the nutritional quality of their diet and that of their families; and an investigation into whether and how the characteristics of so-called 'food deserts' affect the nutritional value of the diets of those who live in areas with limited access to a variety of reasonably priced foods.

6. Comments

Current projects promise to give useful deliverables to meet policy requirements. Researchers are encouraged to focus on delivering practical information on how consumers can best be guided towards a healthy diet and, where possible, tools for disseminating that information.

Dr Jennifer Woolfe of the Food Standards Agency Nutrition Division is the programme manager.

Food acceptability and choice (AN09)

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0918	Parents, Peers and Adverts; the home based interplay of influences on adolescents' dietary habits and attitudes	South Bank University	01/01/97	31/03/99	8,880
AN0921 (N/A)	Development of self-assessments for intake of fat and other nutrients	Birmingham University	01/10/96	30/04/99	14,436
AN0925 (N09001)	A family-based study to determine the acceptability of an increased intake of complex carbohydrates and to explore how change can be achieved	Newcastle University	01/09/97	30/06/02	121,124

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
AN0927 (N/A)	Technical, economic and consumer barriers to the consumption of reduced fat bakery products	Campden & Chorleywood Food Research Association	01/04/98	31/03/99	8,284
AN0928 (N09002)	Barriers to the development and uptake of reduced fat foods	University of Ulster, Coleraine	20/07/98	28/02/01	92,529
AN0929 (N09003)	Development & evaluation of novel school-based intervention to increase fruit & vegetable intake in primary school children	University of Dundee	01/12/98	30/11/00	95,881
AN0930 (N/A)	Food acceptability and choice – research into practice	Glasgow University	01/05/98	30/06/99	6,891
AN0931 (N/A)	Television and food choice (extension of AN0913)	Leicester University	01/02/98	31/01/99	3,551
AN0932 (N09007)	Are fruit tuck shops in primary schools effective in increasing pupils' fruit consumption? A randomised controlled trial	Bristol University	01/10/98	30/09/00	52,571
AN0933 (N09008)	Increasing fruit & vegetable consumption in children: the development & evaluation of a school-based intervention using art/play therapy	Sheffield University	01/10/98	01/10/01	40,001
AN0934 (N09009)	Family centred, school-based intervention for the prevention of obesity in primary school aged children	Oxford-Brookes University	01/07/99	30/06/01	33,216
TOTAL					477,364

Food Safety (MINIM PP1:01)

FS02 (now G01) – Safety of novel foods

1. Background

The EC Regulations on novel foods and food ingredients came into effect in 1997. These require a statutory pre-market safety assessment to be carried out on foods and food ingredients that have not previously been consumed to any significant degree within the EU. The Safety of Novel Foods programme was set up by MAFF to identify, monitor and evaluate the potential risks associated with novel foods and their constituents and to use this information in the safety assessment procedure. This programme is now funded by the Food Standards Agency (April 2000 onwards).

Over the years the research has fallen into a number of broad areas, agro-environmental, analytical, gene expression, gene transfer and general safety issues. Current projects are largely focused on analytical issues, where understanding and improved methodology is needed if safety issues are to be addressed, and on the safety implications of the technology underpinning GM food production. The results are used to ensure that the safety assessment of novel foods reflects the most up-to-date scientific knowledge.

The research programme has been running for seven years. Over this period a total of 23 research projects have been completed, a further 14 are currently under way and another 2 have been approved in principle. To date, over £4.5 million has been spent. In 1999/2000, when 16 projects were in progress, the expenditure was ca. £1.1 million.

2. Outputs

The initial focus of the programme was the generation of well-defined GM lines for analysis and study. The wide range of lines now available can be analysed further and may serve as a resource for future work in other programme areas. Recent highlights of the various programme areas are as follows:

Agro-environmental

A recently completed project has demonstrated that, while it is possible to artificially create hybrids between oil seed rape (*B. napus*) and related weed species (wild radish and charlock), these hybrids were either sickly, sterile

or died. In contrast it was possible to get crosses to related crop plants (*B. juncea* and *B. rapa*). The fate of the transgene and the number of hybrids generated depended on the species used, and the location of the gene in the original GM plant. The viability and vigour of the hybrid varied with the ecotype and parentage, i.e. which plant is the male and female parent. In general the more closely related the species the more hybrids there were formed and, typically, over successive generations the hybrids increased in vigour and the transgene was expressed stably.

In another project chloroplast transformation has been successfully achieved in strawberry. Potentially this approach offers the opportunity to achieve high levels of gene expression without the risk of gene transfer via pollen, a concern within the agricultural environment.

Analytical

A wide range of molecular techniques have been used in the analysis and quantitation of heterologous gene expression. New screening methods are also being developed in this area as the problems of contamination of produce and GM labelling have to be addressed. Two projects are currently underway that involve surveys and questionnaires while another project involves the experimental determination of possible compositional changes following genetic modification, i.e. screening for pleiotropic effects following the insertion of a number of genes. A fourth project involves the evaluation of methods that can be used to establish the range of natural variability within plants so reference standards are available when screening GM plant materials.

Gene Expression

Some of the early observations made with GM plants indicated that not only was the insertion of DNA a random event but that it was less precise than intended, i.e. additional DNA was frequently inserted alongside the desired DNA. In addition, the transgenic plants often displayed unstable gene expression patterns. Studies using GM oilseed rape have demonstrated that, while there is considerable instability in the first generation of transformants, later generations are stable and that, with more careful analysis, many of the apparent expression instabilities can be explained. These studies have also shown the amount of additional DNA inserted varies with the location, species and construct used. Loss of tissue specificity can also be a potential problem. Such studies confirm the need for careful and detailed analysis in the development of GM crops.

Studies with other plant species have also shown that expression levels are more variable in the first generation of GM plants than later ones. In GM tobacco the structure of the construct used had a significant effect on the

translational and transcriptional efficiency of the transgene. Other experiments with GM wheat are also looking at the importance of the transformation technology and the structure of the construct used. To date this has shown that presence of a single intron in the construct boosts expression but the effect varies with the intron type and is reduced by the presence of a second intron. The nature of the construct is clearly important in a variety of ways. Preliminary results from another project have demonstrated that infection of GM plants containing the 35S promoter (which is derived from the cauliflower mosaic virus) with the cauliflower mosaic virus exerts a reduction on the expression of the inserted genes.

On-going studies with GM cereals are looking at the effect of transgene insertion and deletion on the expression of other genes. To date these have shown that expression is higher in homozygous than heterozygous lines. It has also been shown in wheat that the insertion of additional genes that are known to have a role in lipid metabolism not only can affect the nature of the lipids present but also affect plant leaf morphology. In particular the chloroplast size was increased and to a lesser extent mitochondria number. These effects mirrored the normal plant's response to changed environmental conditions where lipid metabolism is again altered.

Gene Transfer

A concern is that the DNA inserted into food materials may be transferred to another organism e.g. gut bacteria. Specific concerns relate to the transfer of antibiotic and herbicide resistance genes that are frequently used as marker genes in the development of GM plants. A number of projects are currently addressing this subject.

Previous work has demonstrated that DNA can survive long enough *in-vitro* in the presence of human saliva to transform some oral bacteria. It has been demonstrated that DNA degradation *in-vivo* is much faster and transformation of oral bacteria is still possible. Certain rumen bacteria are naturally competent and can be transformed by DNA, it is also possible to detect survival of DNA for a short time in the presence of diluted rumen fluids. To date no transformation of gut bacteria by DNA in a colonic fermenter has been detected but it is known that *E. coli* can be transformed *in-vitro* in the presence of calcium ions.

It has been demonstrated that *in-vitro* transfer of DNA into and out of the bacterial chromosome is a relatively efficient process if opportunities for homologous recombination exist. Screening natural isolates of newly identified antibiotic resistance genes has shown that genes from disparate sources have a high level of homology indicating that such transfers occur naturally. The widespread occurrence of natural antibiotic resistance enhances the chances of gene transfer occurring via homologous recombination but also means detection will be more difficult.

Studies with GM maize material containing an antibiotic resistance gene have shown that the material in fact contains two copies of the original gene but that it has been rearranged in the absence of selection pressure and is unlikely to actually confer resistance. Experiments on survivability of plasmid and chromosomal DNA have shown that plasmid DNA can survive for measurable periods in ovine rumen fluids, saliva, and silage effluent. Chromosomal DNA could only be detected for a short period after exposure to saliva but not with the other fluids. The DNA was also capable of transforming bacteria after exposure to ovine saliva but not other fluids. However, mixing DNA and *E. coli* immediately prior to exposure to the silage effluent or rumen fluid led to transformation since the DNA was rapidly taken up by the bacteria.

General Safety Issues

There is increasing interest in probiotics and their presumed health benefits. However there is concern that the consumption of large quantities of live, poorly characterised bacteria could have potential health risks. To test this a variety of strains of *E. faecium* and *E. faecalis* were screened for the presence of specific virulence factors. This showed that strains used as starter organisms for the dairy and food processing industry contained fewer virulence factors than did the commensal bacteria isolated from food. Medical isolates that are opportunistic pathogens contained the most. Some virulence factors were mainly associated with the pathogenic strains and there were also more of these factors in *E. faecalis* than in *E. faecium*. The potential for gene transfer was also demonstrated hence there is the risk that virulence factors could be transferred *in-vivo* to the probiotic after consumption. Consequently, while the dairy starter strains appear safe to use, there is the risk that they could take up virulence factors that would then affect the consumer.

3. Dissemination Activity

Contractors are encouraged to publish their results in peer-reviewed journals although it can be more difficult to publish a negative, albeit desirable, result. Final reports have been lodged in the MAFF library where they can be accessed by the public and are now listed on the Food Standard Agency website. Dissemination of results has also been via posters and presentations since many of the researchers are invited to conferences sponsored by a variety of scientific organisations.

The workshop originally planned for 1999 has been deferred until June 2000 and, in addition to improving communications amongst the contractors, will serve as a useful technical briefing for the Agency staff who will now take responsibility for this programme.

4. Future Activity

Because of the high public profile of the area, the rapidly developing technology underlying the production of GM foods and the public interest in other aspects of novel foods, this is an area that needs further study. It also has to be recognised that risks can and do occur with other non-GM novel foods including novel organic produce, functional foods and both pre- and probiotics.

5. Comments

Despite the high profile of this area and national advertisement of the research requirements, the research remains concentrated in a limited number of laboratories.

The area also remains one of public concern even though the research to date has not revealed the existence of any serious generic problems. Continued thorough analysis and improvements in technology may reduce the risks presently associated with GM foods to the same level of risk associated with conventional foods and various food processing practices.

The programme manager is Keith Cowey, Secretary of the Lister Institute of Preventive Medicine.

FS02 (now G01) – Safety of novel foods

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS0211 (G01001)	Regulation and targeting of transgene expression in fruit crops	Horticulture Research International	01/11/97	31/10/00	86,856
FS0212 (G01002)	Causes of instability in transgenic plants	John Innes Centre	01/09/96	31/08/99	57,313
FS0213 (G01003)	Safety of recombinant DNA technology: gene location, marker elimination and secondary effect	IFR, Institute of Food Research	01/10/96	30/09/99	47,324
FS0219 (G01004)	Persistence and potential infectivity of live bacteria in foods	IFR, Institute of Food Research	01/05/97	31/01/00	20,399
FS0222 (G01005)	The effect of agriculturally-relevant environmental factors on the expression and stability of genes affecting wheat lip	University College Wales, Cardiff	01/07/97	31/12/00	89,249

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS0224 (G01007)	Survival of DNA in the gut and the potential for genetic transformation of resident bacteria	Rowett Research Institute	01/06/98	31/05/01	72,550
FS0225 (G01008)	Evaluating the risks associated with using GMOs in human foods	Newcastle University	01/09/98	31/08/01	116,311
FS0226 (G01009)	Impact of transformation methods, construct & gene cassette architecture on the stability & expression of transgenes	John Innes Centre	01/10/98	30/09/01	82,941
FS0227 (G01010)	Assessment of the risks of transferring antibiotic resistance determinants from transgenic plants to micro-organisms	University of Leeds	01/05/98	30/04/01	80,808
FS0228 (G01011)	Dissemination of GM DNA and antibiotic resistance genes via rumen micro-organisms	Rowett Research Institute	01/06/98	31/05/01	70,899
FS0229 (G01012)	Risk of gene transfer from genetically modified crop plants to gut bacteria	IFR, Institute of Food Research	02/01/99	31/12/01	109,537
FS0230 (G01013)	Causes and consequences of pathogen induced transgene instability	John Innes Centre	01/06/99	31/05/02	54,847
FS0231 (G01014)	Analytical methods for the detection of genetically modified foods – current & future developments	Central Science Laboratory, York	01/08/99	31/01/00	15,240
FS0232 (G01015)	Implications of secondary metabolites produced in response to biotic & abiotic stress by genetically modified potatoes	National Institute of Agricultural Botany	01/06/99	31/05/02	51,849
FS0233 (G01016)	Survey of analytical techniques available & under development for long term monitoring of FS of GMOs and their products	BIBRA Toxicology International	01/04/99	31/08/01	37,860

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS0234 (G01017)	Develop method to study implication of vector insertion/excision for endogenous DNA function in transgenic peas	John Innes Centre	01/10/99	30/09/02	46,705
FS0235 (G01018)	Methods for the study of vector insertion and marker gene excision in a monocot system	John Innes Centre	01/10/99	30/09/02	22,528
TOTAL					1,063,216

FS16 (now A01) – Food additives

1. Background

The purpose of this programme is to assist with the process of ensuring that the use of food additives does not prejudice food safety. Much of the current work aims to develop appropriate methodology to measure levels of additives in foods. This information is needed, for example, to estimate dietary intakes of additives and so help the UK fulfil its EU obligations to provide intake values. The programme also provides underpinning information to assist the UK in EU negotiations on specific topics, for example criteria of purity. Research is not carried out for the purpose of supporting the use of new additives, as the evidence that they are safe and needed must be provided by industry.

The cost of the research for this programme in Financial Year was ca. £503k.

2. Outputs

There has been progress in developing methods of analysis for additives in foods. Research on immunoassay of gum arabic has shown that non-competitive ELISAs are more sensitive but not as specific as competitive ELISAs. Antibodies for carrageenan had different affinities for kappa-, iota- and lambda-carrageenan fractions. Novel chemical techniques also continued to be developed and applied in this programme. Free Flow Electrophoresis is providing promising results in resolving Class III and IV caramels. Capillary electrophoresis-electrospray ionisation-mass spectrometry was applied successfully to analyse food products spiked with gellan gum. A feasibility study is being carried out on the use of gas chromatography-combustion-isotope ratio mass spectrometry to distinguish between benzoates that are naturally present in food and those used as additives.

The use of biomarkers to help in estimating dietary intakes of additives has been researched further. It has proven difficult to identify urinary metabolites that can be used as measures of intake for caffeine, although three of its metabolites are being studied further (1,7-dimethyl xanthine, 1,7-dimethyl uric acid and 1-methyl xanthine).

A Unified Food Additives Information System is being developed to draw together information in databases produced over the years. A literature review has also been carried out on additive-additive and additive-food component interactions. The main conclusions were that international research on this topic would benefit from more co-ordination, and it is important to understand the parameters that dictate the outcome of additive interactions.

3. Dissemination Activity

A project review meeting was held in November 1999 where the results of recent research in this programme were presented by project leaders and considered by the Working Party on Food Additives. A booklet containing summaries of the projects and the Working Party's discussions was produced and distributed. This review provided valuable information to assist in interpreting results. A further review meeting will be held in November 2000.

During the course of the year, 11 papers were published on the results of this programme and 6 presentations were given at scientific meetings.

4. Future Activity

In Financial Year 2000/01 much of the work summarised above will continue. The Unified Food Additives Information System will be developed further in a new project. There will be a new project on development of methods of analysis for antioxidants. Further work on immunoassays for additives is being carried out on thaumatin. Work started recently on a single validated chemical method of analysis for the simultaneous determination of intense sweeteners in foodstuffs.

The programme will be subjected to regular review.

5. Comments

The contact point for information about this programme is Mr Mark Willis of Chemical Safety and Toxicology Division, Branch 4.

FS 16 (now A01) – Food additives

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS1632 (A01003)	Development of an immunoassay for gum arabic	North East Wales Institute	01/07/95	31/03/99	16,471
FS1639 (A01005)	Novel approaches to the identification and quantification of caramels in foods	Leatherhead Food Research Association	01/06/97	31/05/00	70,710
FS1642/1654 (A01006/A01017)	Dietary determinants of sulphide production in the human large intestine	Dunn Clinical Nutrition Centre/ University of Dundee	01/10/97	30/09/99	33,892
FS1643 (A01007)	Urinary biomarkers for assessing caffeine intake	Central Science Laboratory, York	01/04/97	31/10/00	123,770
FS1646 (N/A)	An improved analytical method for gellan gum	Laboratory of the Government Chemist	01/06/98	31/03/99	12,885
FS1647 (A01010)	Development & validation of procedures to detect & quantify the use of dimethyl dicarbonate in non-alcoholic drinks	Laboratory of the Government Chemist	01/09/98	31/08/99	39,899
FS1649 (A01011)	Development of immunoassays for quantification of thaumatin/alginate in foods	South Manchester University Hospitals Trust	01/06/99	30/08/01	17,053
FS1650 (A01012)	The development and validation of an HPLC method for the simultaneous determination of intense sweeteners in foodstuffs	Central Science Laboratory, York	01/07/99	31/01/01	45,710
FS1651 (A01013)	Biomarker assessment of benzoate consumption	Laboratory of the Government Chemist	01/05/99	31/03/00	26,540
FS1652 (A01014)	A unified food additives information system	AEA Technology	01/04/99	31/03/00	24,640
FS1653 (A01015 & A01016)	Validation of ELISAs for the detection and quantification of gums in foodstuffs	University College Chester/ Leatherhead Food Research Association	01/06/99	31/12/00	91,784
TOTAL					503,354

FS17 (now T01) – Risk assessment

1. Background

The Risk Assessment Programme aims to refine techniques used in the assessment of risk from chemicals in food and include considerations of variations in susceptibility of different groups in such assessments. Elements of risk assessment include identification and evaluation of hazards, and estimation of intake from an assessment of consumption and exposure. Conventional animal toxicology studies are based on testing of single compounds in isolation at doses greatly in excess of those found in the human diet. A particular emphasis of the Risk Assessment Programme is the development of models that are more appropriate to underlying biological processes in humans. The uncertainties involved in extrapolation between species and from test doses to relevant doses found in the normal diet, and the potential interactions with synergistic or protective factors within the food matrix need to be taken into account. Therefore the scientific objectives of the programme are to:

- (i) develop approaches to food risk assessment which take into account variability and uncertainty;
- (ii) develop integrated approaches which take due account of human data, *in vivo* data, *in vitro* data and expert judgement as appropriate;
- (iii) continue the development, where appropriate, of biomarkers of exposure in the context of the development of exposure assessment methodology;
- (iv) review and develop the methodology for exposure assessment for food chemicals, including exposure assessment for population subgroups, and the integration of the hazard assessment with the exposure assessment into an overall risk characterisation.

A primary focus of this research programme is on risk assessment for genotoxic carcinogens that occur unavoidably in foodstuffs. The rationale for this is two-fold:

- Currently accepted risk assessment methodology does not support identification of a level of exposure to genotoxic carcinogens that is without risk; and
- it is widely accepted that about one third of human cancers are of dietary origin, whilst some dietary components have been shown to decrease risk of cancer.

Together, these considerations form a compelling requirement for advancing the scientific understanding of exposure and response to chemical carcinogens associated with the diet, as well possible interactions with dietary factors that are thought to reduce the risk of cancer.

Other areas of focus relate to:

- Development of a more scientific basis for the risk assessment of non-carcinogenic food chemicals (for example, the use of safety factors applied to results of animal experiments);
- Development of methods that minimise animal experimentation, in accordance with ethical and legal requirements.

The cost for this programme in financial year 1999/2000 was *circa* £1.4 million.

2. Outputs

Modelling of intake and internal exposure

In June 1998, the participants of a workshop entitled “Probabilistic Approaches to Food Risk Assessment” (funded under the MAFF FS17 research programme), identified the potential value of probabilistic methods for improving intake estimation for food chemicals. As a result, two projects were commissioned in 1999, which are using complementary mathematical approaches in order to derive quantitative estimates of the uncertainty and variability associated with an individual’s intake of food chemicals and patterns of intake for different individuals including day-to-day variability. The projects are using data from the Dietary and Nutritional Survey of British Adults, 1986–87 to develop and explore the methodology and will increase understanding of the variability and uncertainty in the range of intakes of food chemicals within the UK population.

Subsequently, a project has recently been commissioned to develop biomarkers of internal exposure (internal dose). Data on these biomarkers will be used to analyse the appropriateness of the default inter- and intra-species safety factors in relation to the fate of food chemicals in the body.

These projects are addressing objectives (i) and (iv).

***In vitro* approaches to food chemical risk assessment**

The Food Standards Agency is part-funding an EU Framework IV collaborative research project (EUROSLICES) which aims to optimise conditions for maintaining and preserving precision-cut liver, kidney and

lung slices of human and rat origin, facilitating inter-species comparison of metabolism and toxicity. The project has resulted in marked improvement in the preservation of metabolising capacity in cryopreserved liver slices, which will ensure that maximum research benefit can be derived from human tissue which is infrequently available.

A second *in vitro* project will generate cell lines that simulate the human intestinal barrier in order to investigate bioavailability of constituents within the food matrix. So far, the project has generated baseline information that will enable further development of the model to assess active and passive transport of selected biologically active food components. Data from these studies will be compared with human *in vivo* data to validate the technical approach.

Establishment of reliable *in vitro* techniques will support future development of objective (ii).

Dietary constituents and colorectal cancer

A collaborative study of the possible involvement of heterocyclic amines (HA's) in causation of human colorectal cancer was initiated in 1997 and is now nearing completion. Large numbers of colorectal cancer patients and matched controls have been recruited and data are being collated on dietary histories, ability to form the carcinogenic metabolites of heterocyclic amines and occurrence of the characteristic mutational "fingerprint" that is associated with heterocyclic amine exposure. Scientific rigour requires that the data cannot be decoded and analysed at this stage, but the study is on course to provide valuable information. This includes:

- Genetic studies of a range of carcinogen metabolising enzymes may help to explain different individual susceptibilities to colorectal cancer.
- The study may identify specific components of the diet which are of particular risk factors in the development of colorectal cancer or, conversely, may be protective against the disease.
- The technique of Accelerator Mass Spectrometry (AMS) has enabled the detection of ¹⁴C DNA adducts following the administration of low doses of ¹⁴C labelled compounds to humans.
- The study has shown that the major heterocyclic amines, MeIQx and PhIP, are bioavailable to the human colon where they can form DNA adducts. Large interindividual variation is apparent in DNA adduct levels for both compounds which will

be compared with the individuals' phenotype/genotype for the specific drug metabolising enzymes, when the data are decoded.

- Analysis of mutation spectra in a number of genes which have been associated with the pathogenesis of colorectal cancer will determine whether the observed mutation spectra are different in individuals exposed and not exposed to dietary heterocyclic amines.

Collectively, these studies should support a comprehensive assessment of the relevance of the heterocyclic amines to diet associated human cancer, in support of objectives (ii) and (iii).

Related studies have shown that:

- Consumption of a diet with high levels of cruciferous vegetables (Brussel sprouts and broccoli) altered the disposition of heterocyclic amines (HA's) in humans in a well-controlled intervention study, in a manner compatible with induction of HA metabolism.
- Administration of Brussel sprout extract to rats and mice resulted in induction of a number of glutathione S-transferases and quinone reductase in small and large intestine, but had little effect on cytochrome(s) P450 expression, suggesting that Brussel sprout extract may retain the anticarcinogenic properties of cruciferous vegetables. Studies in humans are now in progress.

Biomarkers of effect in carcinogenesis

A number of new projects have been commissioned relating to use of biomarkers in risk assessment of dietary genotoxins, in response to objective (iii):

- Comparison of DNA damage and repair in target versus non-target cells will aim to establish a better correlation between adduct levels and risk of tumorigenicity, providing an enhancement of safety/risk assessment procedures, and elucidate the mechanisms linking lesion induction and tumorigenesis.
- Novel biomarkers, based on cell cycling and proliferation, will be developed to assess whether they are predictive of subsequent tumour formation in experimental animals, and can

be used in conjunction with biomarkers of exposure to provide a better assessment of the hazard of food and environmental chemicals to humans.

- A study of stem cell mutational indices aims to link measurements of mutagen exposure (DNA adduct levels) to biomarkers of effect (mutational indices) in a new short-term bioassay for carcinogenic potential of food components. The bioassay will quantitate effects of both dietary mutagens and of dietary non-genotoxic agents that act through various epigenetic pathways to increase the total load of mutated stem cells.
- A study of combined effects of a dietary genotoxin and oxidative DNA damage, which may result from endogenous processes, aims to investigate the role of DNA repair in the dose-response relationships.

Whole food approaches

A recently completed project on biomarkers for reactive nitrogen species generated a number of new methods and contributed to the fundamental understanding of the production and reactions of reactive nitrogen species:

- Considerable data were produced on levels of nitrate and nitrite in a range of foods, and in human body fluids. Modification of dietary intake of nitrate and nitrite resulted in changes in the levels of these materials in the human body.
- Flavonoids and phenolic substances in plants were shown to inhibit formation of reactive nitrogen species, but nitrated flavonoids were not sufficiently specific to be used as biomarkers of scavenging for reactive nitrogen species.
- Existing methods for measuring nitrated tyrosine residues in protein were shown to be inaccurate, raising concern over the accuracy of previously published data. A new robust method for measuring total (free plus protein-bound) nitrotyrosine was developed.

The Food Risk Assessment Programme (FORA) aims to develop methodologies for quantitation of exposures to, and biological effects of, mutagens associated with diet. Progress has been made in a number of areas:

- Malondialdehyde-DNA adducts could be detected in approximately half of the gastric biopsies collected from 40 patients recruited into a *Helicobacter pylori* eradication clinical trial. Levels of adducts were not affected by *H. pylori* infection or measures of lipid peroxidation.
- The levels of malondialdehyde-DNA adducts in blood DNA were increased in volunteers eating meat but in a non-dose-dependent manner and was dependent on cooking method.
- Biopsy samples from participants in the European Prospective Investigation on Cancer study (EPIC) have been analysed for the presence of malondialdehyde-DNA adducts and results are awaiting statistical analysis.

The FORA programme also includes a number of workshops designed to explore specific issues related to risk assessment for whole food. The third workshop – “Diet-gene interactions: Characterisation of risk” – was held in February 2000. The workshop identified encouraging new opportunities, including the use of developing molecular technologies, to advance investigations into the way genetics and diet may interact to modify cancer risks. The fourth workshop – “Chemoprevention and chemoprotection: the role of dietary intervention and how to measure its effects” – was held in June 2000.

3. Dissemination Activity

Annual workshops of the Risk Assessment Research Programme were held on July 6/7 1999, at Christ’s College Cambridge, and on June 27/28 2000, at the Hilton Hotel, Stansted Airport.

The first two reports of the FORA workshops have been published – “Probabilistic approaches to food risk assessment (FORA 1)” and “Energy metabolism and carcinogenesis (FORA 2)” and are available from the MRC Institute of Environment and Health. A large number of high quality scientific publications and presentations have resulted from the research in this programme.

4. Future Activity

The research has resulted in identification of a number of related research needs and priorities. These have been incorporated into Research Programme T01 of the Food Standards Agency Research Requirements Document for potential funding in 2001.

5. Comments

The Programme Adviser is Dr Diane Benford.

FS17 (now T01) – Risk assessment

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS1729 (T01001/2)	Biomarkers of reactive nitrogen species: their role in genotoxicity and the effect of diet	United Medical & Dental School Kings College, London	01/10/96	30/09/99	97,112
FS1731 (T01003)	Can Biomarkers be used to assess the carcinogenic potential of heterocyclic amines	Imperial College, London	01/06/97	30/09/00	107,667
FS1732 (T01004)	Heterocyclic amines as risk factors in colon cancer	University of Dundee + subcontract with Leeds*	01/06/97	30/12/00 (*ends 31/03/00)	206,598
FS1733 (T01005)	Measurement of the formation of ME1Qx and PhIP DNA adducts in human colon cancer and non cancer patients	University of York	01/06/97	30/03/00	148,898
FS1735 (T01007)	Food Risk Assessment Fellowship	MRC Toxicology Unit Leicester	01/09/97	31/12/00	258,093
FS1736 (T01008)	Development of an in vitro intestinal cell model to predict bioavailability of food components in humans	Rowett Research Institute, Aberdeen	01/04/98	30/04/02	52,862
FS1737 (T01009)	Can cruciferous vegetables alter the genotoxicity of heterocyclic amines following human consumption of cooked meat?	Imperial College, London and TNO-BIBRA International	01/06/98	30/09/01	223,380
FS1738 (T01010)	Cruciferous vegetables and drug-metabolising enzyme phenotype	University of Dundee	01/04/98	30/09/00	76,332
FS1739 (T01011)	New developments of cultured precision-cut tissue slices for studies of organ pharmaco-toxicology	TNO-BIBRA International	01/08/98	31/03/01	42,785

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS1740 (T01012)	The interactions of genotoxins – a mechanistic approach based on DNA repair	Central Science Laboratory/ Institute of Food Research	01/04/99	31/03/02	131,880
FS1741 (T01013)	Development of probabilistic models for describing individual intakes of chemical residues in food	TNO-BIBRA International	01/04/99	31/03/01	51,520
FS1742 (T01014)	Modelling of inter- & intra- individual exposure to chemicals in food	MRC Biostatistics Unit, Cambridge	01/04/99	30/09/01	19,021
TOTAL					1,416,148

FS18 (now D01) – Risk management (now Risk communication)

1. Background

The Risk Management Programme was initiated on 1 April 1994, though some projects had been commissioned earlier (dating from 1991). The objectives of the programme were:

- To improve the safety of the food supply and to develop a rational and cost-effective framework for the management of food-related risk;
- To investigate, and where appropriate develop, alternative strategies for managing food chemical contaminants, in particular techniques for balancing risks costs and benefits;
- To investigate sources of influences on consumers' perceptions of food-related risks, and to develop techniques for monitoring perceptions and their influence on behaviour and to develop strategies for introducing this information into the risk management system; and,
- To investigate the factors which influence the efficacy of food-risk communications and develop improved communication strategies.

In 1998, following a major review of the programme, it was decided to focus further upon communication issues (no longer focusing primarily or solely

upon risk issues but considering all aspects of communication concerning food) and the Communication research programme was established. Its objectives are:

- To inform the development of the communication strategy of the Food Standards Agency;
- To identify how to communicate food-related health information more effectively.

Most of the outputs reported here were generated under the aegis of the earlier Risk Management programme. Two projects have been commissioned under the Communications programme but these have only very recently begun. One of these is examining how best to achieve multiple stakeholder participation and the other is exploring the dynamics the use of partnerships to promote food safety and nutrition messages. Expenditure for this project in FY 1999/00 was *ca.* £224k.

2. Outputs

In examining outputs, it is necessary to cover those of the Risk Management programme and the new Communications programme. The main achievements of the Risk Management programme to date are:

- valid and reliable methods for monitoring public and expert knowledge of and concerns about food hazards;
- descriptions of the patterns of public food risk perceptions and beliefs and assessments of their responsiveness to new information;
- predictive models of the factors influencing risk reactions and response to risk communications;
- identification of the role of source and context in determining impact of risk communications;
- frameworks for assisting decision-making by policy makers.

The programme was characterised by:

- a focus on specific hazards – the early studies examined food risk perceptions in a broad way, recent studies have devoted attention to specific hazards – recognising that risk management strategies may need to be customised for specific hazards;

- a focus on intervention methods not just monitoring – studies have not simply tried to describe baseline patterns of risk perceptions, they are designed to help to identify the methods that might be used to intervene to change beliefs;
- research conducted by multi-disciplinary teams;
- researchers who engage consultation with MAFF to ensure that their studies are relevant;
- studies with design rigour and novelty;
- cross-project liaison and data-sharing.

In the course of the year four projects under the Risk Management programme were successfully completed, they were:

- a comparison of different sampling techniques (multi-stage/sentinel site/internet using postal questionnaires, telephone interviews, and e-mail) to assess public perception of food risk – designed to allow the development of effective surveillance methods;
- an analysis of the types of information people receive from the media about food hazards (including GMOs) and how they react to those messages
- an assessment of the impact of information content and presentational context upon perceptions of specific food risks (including GMOs, BSE, Salmonella, pesticide residues in food, and high fat diet);
- the creation of a database which summarises the details of all researchers currently engaged in research on communication concerning food-related issues.

One project from the Risk Management programme is currently ongoing: examining how risk uncertainty is understood by the public and scientific experts.

Two projects under the Communication programme have begun. They entail:

- analysing Food Standards Agency interactions with stakeholders to help to achieve open and transparent mechanisms for communication;

- assessing how to develop approaches to risk communication that optimise communication partnerships to ensure that the message is delivered from the most effective source in the most effective way to ensure message take-up.

3. Dissemination Activity

Projects commissioned in the Programme regularly report results in peer-reviewed academic journals and at conferences. In addition, there have been briefing meetings with various groups with policy responsibilities.

The Programme Adviser organised the annual workshop for the programme in London in February 2000.

4. Future Activity

The programme will continue to commission research capable of contributing to developing a communication strategy. Specifically, issues concerning the problems of communicating with socially excluded groups and the role of “pressure groups” have been considered for examination. Also, it is felt important that the tracking of consumer perceptions and behaviour over time, in relation to media influences, would be valuable in developing an issues identification and management tool necessary for the Food Standards Agency. Several new food-related hazards are now possible foci for research: for example, anti-biotic-resistant micro-organisms and certain novel foods.

5. Comments

It is vital to translate research findings in this area into a form which can be useful for policy development and implementation. Greater effort must be put into this. In commissioning research, it is now clear that this is a requirement.

The nature of the project commissioning process adopted previously tended to result in diversity in project methodologies and theories but militated against coherence and linear development of the research base. Some changes were needed in this process. Consequently, it has been decided that in the Communication programme there will be greater co-ordination of individual projects and that clusters of projects (involving multi-site, multi-disciplinary collaborations) will be encouraged.

The Programme Adviser is Professor Glynis M Breakwell, University of Surrey.

FS18 (now D01) – Risk management (now Risk communication)

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS1844 (D01001)	The impact of information content and presentational context on perceptions of specific food risks	IFR, Institute of Food Research	01/04/97	31/03/00	72,536
FS1845 (D01002)	Information sources and their influence on public perceptons of food hazards	Glasgow University	01/06/97	30/09/99	35,666
FS1847 (D01004)	Comparison of different sampling techniques to assess public perception of food risk: Development of a surveillance approach	University of Leeds, Division of Public Health	01/05/98	30/04/00	33,559
FS1849 (D01005)	Communicating risk uncertainty with the public	IFR, Institute of Food Research	01/06/99	31/12/00	26,458
FS1849 (D01006)	Communicating risk uncertainty with the public	Newcastle University	01/06/99	31/12/00	25,443
FS1850 (D01007)	Social Amplication of Risk	Health and Safety Executive	01/04/99	31/12/00	30,000
TOTAL					223,662

FS19 (now A05) – Food irradiation: Research in support of detection tests and the provision of scientific advice**1. Background**

Food irradiation is currently regulated under The Food (Control of Irradiation) Regulations 1990. The regulations provide for the licencing of irradiation facilities to process seven food categories. The Food Labelling Regulations 1996. requires all irradiated foods to carry an indication of such treatment.

In February 1999 the European Union and the European Parliament published two EC Directives on foods and food ingredients treated with ionising radiation. All member states are requires to implement these directives by September 2000. EC Directive 1999/2/EC lays down general provisions (i.e. conditions for treatment, rules governing the approval and the control of irradiation facilities and labelling) and EC Directive 1999/3/EC establishes an initial positive list of foodstuffs (a list of foods that may be irradiated and freely traded across the European Community). These

Directives will be implemented in England by the Food Irradiation Provisions (England) Regulation 2000. This new regulation will amend the existing Food (Control of Irradiation) Regulations 1990.

The detection test research programme has had considerable success. Validated methods for detecting a variety of irradiated foodstuffs have been published. The ultimate objective is to produce a battery of tests and screening tests that can be used by enforcement officers. Current objectives are focussed on developing and validating detection tests for high value foods likely to be commercially irradiated (e.g. products such as spices, seafood and to a lesser extent fruit and vegetables).

The total spend in FY 1999 – 2000 was £70.4k.

2. Outputs

To date four fully validated tests have been published in the Journal of the Association of Public Analysts; These test are (i) the detection of irradiated herbs and spices using thermoluminescence (TL), (ii) detection of bone-containing meat using electron spin resonance (ESR) spectroscopy, (iii) detection of food containing fat using 2-alkylcyclobutanone markers and (iv) poultry meat using the Limulus Amoebocyte Lysate (LAL) test in conjunction with a Gram negative bacterial count (GNB). The first three of these methods were successfully used in a MAFF authenticity survey for irradiated food undertaken in autumn 1996.

Several more methods have been successfully validated by international collaborative trials; (i) microgel electrophoresis (a screening method to detect irradiated chicken, pork and plant seed); (ii) the Direct Epifluorescent Filter Technique/Aerobic Plate Count (DEFT/APC) screening test for detection of a range of irradiated foods (including prawns, cod, beefsteak and minced beef); (iii) the photostimulated luminescence (PSL) method for detection of irradiated shellfish and (iv) PSL detection of irradiated herbs and spices; (v) the thermoluminescence (TL) method for the detection of irradiated shellfish, and (vi) TL detection of irradiated fruit and vegetables. Publication of these six validated methods should take place once peer-review of the collaborative trial reports has been completed satisfactorily.

The completion of two separate collaborative trials, aimed at validating the following methods; (i) the 2-alkylcyclobutanone method for the detection of irradiated exotic fruit, camembert cheese and salmon; and (ii) an Enzyme Linked Immuno-Sorbent Assay (ELISA) capable of detecting radiation-induced DNA base changes in shrimps and prawns, has been delayed. Both will continue in 2000. A rapid 'dip-stick' screening test for the detection of irradiated fat-containing food, based on an ELISA capable of detecting 2-alkylcyclobutanones, has been further developed but this method is not yet

at a stage where it could be validated by collaborative trial. In recognition of the potential problems caused by the blending of irradiated and unirradiated spices, a study to conduct a systematic evaluation of the effectiveness with which the current validated luminescence methods detect such blends was carried out. The results of this study were used to guide development work.

3. Dissemination Activity

Results from the projects have been published in internationally recognised scientific journals. Project reports are made available from the in-house library and publicised in the Food Surveillance Information Bulletin. Food irradiation detection test seminars were held annually until 1997. The research programme is now consolidating and methods are being refined, consequently seminars are held less frequently. Seminar proceedings have been published in the open literature (for example the 1997 seminar proceedings were published in Food Science and Technology Today Volume 12, No.2, June 1998). In addition successful collaborative trial reports and protocols for 6 additional validated methods are due to be published in the Journal of the Association of Public Analysts.

To date five protocols for EU Standards on the detection of irradiated food have been accepted by CEN and, subsequently, by the British Standards Institute (BSI). Work carried out by programme contractors has contributed to these protocols (those based on 2-alkylcyclobutanones, TL and ESR methodologies). Draft EU Standards based on successful collaborative trials of the PSL, DEFT/APC and LAL/GNB screening methods, as well as an extension of the TL methodology to cover shellfish, fruit and vegetables have been put forward for adoption by CEN.

4. Future Activity

The development of statistical and advanced instrumental PSL/TL techniques is on-going at the Scottish Universities Research And Reactor Centre. This research follows on from previous work to evaluate the effectiveness of the current TL and PSL methods.

Detection of irradiated fat-containing foods by the analysis of alkylcyclobutanones, is being developed at the University Of Westminster. The aim is to provide a fast and cheap method of identification by using a rapid fat extraction method in conjunction with Thin-Layer Chromatography.

Emphasis will be placed on advancing those methods that are ready for, or have completed, validation by collaborative trial to ensure that they are published and provide food law enforcement officers with a comprehensive battery of validated detection tests for irradiated foods.

5. Comments

The programme manager is Mr Paul Holley.

FS19 (now A05) – Food irradiation: Research in support of detection tests and the provision of scientific advice

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS1917 (N/A)	Studies to develop a rapid screening test for irradiated food	Queen's University of Belfast	01/10/96	31/03/99	8,096
FS1924 (A05003)	Validation of an ELISA to detect irradiated food by inter-laboratory trial	North East Wales Institute	01/04/98	30/06/99	28,812
FS1926 (A05005)	Trials using new DSE method for the analysis of cyclobutanone in a range of lipid containing irradiated foods	Westminster University	01/07/99	31/10/00	20,126
FS1927 (A05006)	Investigate statistical & imaging methods for luminescence detection of irradiated ingredients in blended foods	Scottish Universities Research and Reactor Centre	01/10/99	30/09/01	13,372
TOTAL					70,406

FS20a (now T05) – Phytoestrogens in the diet

1. Background

Phytoestrogens are naturally occurring compounds found in plants with structural similarities to the female sex hormone, oestradiol-17 β . Because of this structural similarity, concern has been expressed that ingestion of phytoestrogens might have oestrogenic effects which could be both beneficial and/or detrimental and could target particular groups in the population. There are a variety of these plant oestrogens – lignans, isoflavones, coumestans and flavones, all of which bind with varying degrees of affinity to the oestrogen receptors. While it has been assumed that any oestrogenic effects will be exerted through the oestrogen receptor, there is the possibility that phytoestrogens may alter oestrogen metabolism or, for example, act as antioxidants (i.e. flavones). The Phytoestrogen Research Programme is mainly concerned with isoflavones, as these are present in soya based foods which are widely consumed in the UK. Future studies are however being directed towards the lignans and coumestans.

The Phytoestrogen Programme arose from studies being carried out in the MAFF Natural Constituents of Food Programme and became a separate Programme in 1997. The research carried out is determined by 9 scientific objectives laid out in the ROAME A which are designed to provide answers to three Policy objectives. In summary these scientific objectives encompass the development of suitable analytical methods, the determination of phytoestrogens in food, studies on the absorption, metabolism and excretion and possible beneficial and/or detrimental effects in the population and specific (genetic and/or age) groups within the population. The studies are designed to provide information to allow balanced judgements to be made about the risk/benefit of consuming phytoestrogen containing foods and allow meaningful advice to be given to the consumer.

At the time of writing, there are 25 projects under way, some of course coming to an end (around 4) while others are just beginning (4) at a total cost in 1999/2000 of approximately £734k. At the beginning of FY 2000/01, responsibility for this research moved from MAFF to the Food Standards Agency.

2. Outputs

It has only been possible to develop a co-ordinated and comprehensive approach to this increasingly important area of research since the Phytoestrogen Programme was started in 1997. Three areas of interest have been studied, (a) analytical methodology designed to develop reliable and accurate methods for the measurement of isoflavones in body fluids as well as in food, (b) scientific studies to learn more about the effects of phytoestrogens *in vitro*, and (c) studies to evaluate the beneficial and/or detrimental effects using *in vivo* studies on human volunteers. While animal studies have been undertaken in some contracts, it is recognised that public opinion is against the unnecessary use of animals in toxicity testing and strenuous efforts have been made to take account of this, ensuring that such experiments are carried out only when absolutely necessary. All studies on humans have been subjected to careful scrutiny by both MAFF/Food Standards Agency and local ethical committees and informed consent has been obtained from all patients/subjects involved. It is not possible to detail all the work which has been carried out in such a large programme but highlights in each area are:

(a) Analysis

- A major problem in this area has been the lack of standards, both labelled and unlabelled and to address this a major contract was set in place to remedy these deficiencies. This contract has been a major success producing high quality material which in many instances is not available from any other source world-wide. Considerable interest has been shown in

these products and it may be possible to market them which may recoup for MAFF/Food Standards Agency some of the expenditure in supporting this work. Stable isotope labelled isoflavones are now available which have been used for *in vivo* human metabolic studies as well as internal standards for mass spectrometric analytical methods. This contract underpins all the research in this programme and provides necessary material for other contractors. One contractor is studying the possibility of using soya plants grown in $^{13}\text{CO}_2$ as a source of labelled phytoestrogens.

- Gas chromatography-mass spectrometry (GC-MS) has been widely used for determination of isoflavones but more simple methods such as liquid chromatography (LC) have been used to determine concentrations of isoflavones in infant formula feeds. GC-MS has been used to provide concentrations of genistein and daidzein in 200 foods giving a useful database which can be used by other contractors. Phytoestrogens are also present as glycoside conjugates and studies have been undertaken to develop LC-MS methodology which can measure these glycosides directly. This methodology has been applied in a short-term study to determine phytoestrogen concentrations in over the counter preparations available to the public. Further method development continues and is now being extended to the lignan group of phytoestrogens. Measurement of phytoestrogens in human body fluids is important as a means of assessing compliance and to ensure consistency between contractors, a quality assurance scheme was instituted in 1997. A recent survey showed surprisingly good agreement between contractors and the intention is to continue this.

(b) *In vitro* and animal studies

- Infants and neonates who are fed on soya based formula are a particular group of interest and studies continue to ascertain the possible effects of phytoestrogens on the expression of oestrogen responsive genes. A new project to study this *in vitro* has just started using modern techniques of molecular biology together with cDNA microarrays and proteomics. Parallel studies by the same contractor have identified oestrogenic components of soya-based infant formula which cannot be correlated with known phytoestrogen components. Gender differences in the metabolism of genistein in rats have also been identified by a contractor together with possible concentration of this isoflavone in reproductive tissues. Further studies continue in this complex area.

- Functional assays for oestrogenicity are also being developed, primarily using the oestrogen receptor (ER) alpha but recognising the importance of ERbeta, it is intended to incorporate this newly recognised receptor into these studies.

(c) In vivo human studies

● As part of a long-term major study of breast cancer (EPIC) funded *inter alia* by the MRC, several contractors are studying the effects of phytoestrogens on osteoporosis, and prostate and breast cancer. No results are yet available as this is a double blind retrospective study. However preliminary data seem to indicate a protective effect of phytoestrogens in pre- and peri-menopausal women. Studies on the effects of phytoestrogens in men are also being undertaken, one which has now been completed indicates no observable short-term effects on cardiac markers while the other is an epidemiological study, results from which will be available towards the end of this year.

● Further studies which are underway concern the ADME of isoflavones and the effects of diet, and gut microflora on bioavailability in adults and children. Results from these studies are not yet available.

3. Dissemination Activity

● The annual Workshop of the Programme was held in Coventry in September 1999. All contractors attended and presented reports on their progress. The Workshop was attended by invited experts from UK, Europe and the USA who were invited to comment on the research carried out within the Programme

● Contractors are encouraged to present their work by attendance at scientific conferences and by submission of research paper to journals. Contractors have been asked to let the Programme Advisor know of their publications, and so far there have been 12 papers published in peer-reviewed journals, 2 manuscripts submitted, 6 papers in preparation and 4 abstracts or talks.

● All contractors are obliged to deposit final reports in the Food Standards Agency library and these can be accessed through the Agency Web page

4. Future Activities

● Research in the phytoestrogen area is at present under consideration by a Working Group of the Committee on Toxicity, whose recommendations will clearly have a major impact on the future of this programme.

● In tandem with the deliberations of the COT Working Group, a comprehensive evaluation of the Programme is being undertaken by Dr. Margaret Ashwell (UK) and colleagues, and Professor Stephen Barnes (USA). Contractors and external international experts will be evaluating the programme and there will be a formal Review towards the end of this year.

The Review which will be held in Cambridge will be attended by members of the COT Working Group and will form part of their deliberations.

- New contracts which start this year or have just started involve looking at the involvement of phytoestrogens in oestrogen metabolism in blood cells, the effects of lignans on post-menopausal symptoms, use of modern techniques of molecular biology & proteomics to study expression of phytoestrogen responsive genes in neonatal cells and further retrospective studies using the EPIC cohort.
- The June edition of the Food Standards Agency Research Requirements Document 2001/2002 includes a request for proposals to continue to provide a suitable Quality Assurance scheme to monitor contractors' performance and to continue synthetic chemistry to provide labelled and unlabelled phytoestrogen standards. These two areas are essential for the continuing success of this research programme. The final call for proposals recognises the need to extend existing studies using *in vitro* assay systems to utilise the ERbeta.

5. Comments

The Programme Advisor is Professor Hugh Makin

FS20a (now T05) – Phytoestrogens in the diet

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS2060 (T05001)	Synthesis of labelled and unlabelled isoflavonoid phytoestrogen standards	St Andrews University	01/05/96	30/04/02	125,494
FS2067 (T05002)	Dietary phytoestrogens; possible beneficial and adverse effects in men	Rowett Research Institute	01/02/97	30/06/00	32,556
FS2069 (T05003)	Dietary phytoestrogens: possible effects on prostate cancer and 5-alpha reductase activity	Edinburgh University	01/06/98	31/05/01	32,235
FS2071 (T05004)	Effects of phytoestrogens and related dietary components on bone metabolism	Rowett Research Institute	01/02/97	31/01/00	52,200
FS2073 (N/A)	Absorption, distribution, metabolism and excretion of [¹⁴ C] labelled genistein	Veterinary Laboratories Agency	01/04/97	01/04/00	50,564

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS2075 (T05005)	Development & application of screening assays for the beneficial & adverse effects of phytoestrogens in food	Veterinary Laboratories Agency	01/04/97	31/03/00	57,631
FS2076 (T05006)	Investigation of the post-natal developmental toxicity of isoflavones in rats	ZENECA Specialities	01/05/97	01/03/00	71,258
FS2083 (T05019)	The absorption, distribution, metabolism and excretion of isoflavones <i>in vivo</i>	Surrey University,	01/01/98	30/09/99	53,462
FS2085 (T05009)	The use of biologically produced 13C enriched isomers of the phytoestrogens for use as analytical standards	Dunn Clinical Nutrition Centre	01/12/97	30/11/99	29,842
FS2086 (T05010)	Absorption & metabolism of dietary phytoestrogens in humans – effect of age, gender, food matrix & chemical composition	Surrey University	01/07/98	30/06/01	129,455
FS2087 (T01012)	Influence of human gut microflora on dietary soya isoflavone phytoestrogen bioavailability in adults and children	University of Ulster	01/10/98	30/09/01	16,351
FS2087 (T05011)	Influence of human gut microflora on dietary soya isoflavone phytoestrogen bioavailability in adults and children	Kings College, London	01/10/98	30/09/01	82,887
TOTAL					733,935

FS20b (now C03) – Reduction of mycotoxins in foodstuffs and animal feeds

1. Background

Mycotoxins are toxic substances produced by a range of moulds, growing on food crops in the field and in storage. The growth of moulds and possible mycotoxin contamination of foodstuffs is generally favoured by humid conditions at warm or ambient temperatures. A programme has been set up to reduce the levels of mycotoxins in foodstuffs. The programme aims are:

- To develop further, an understanding of the conditions that facilitate the production of mycotoxins in foodstuff during cultivation, harvesting and storage.
- To develop a code of Good Manufacturing Practice (GMP) in order to reduce consumer exposure to mycotoxins and to enable regulatory limits to be met.
- To identify and investigate any new or potential problems caused by mycotoxins.
- To develop a new and improved method of detecting mycotoxins in food.
- To produce results which can be used to influence EU and other international bodies, in agreeing limits for mycotoxins.

The programme started off in 1996 with four projects. The status of each project is summarised below.

The cost of this programme in Financial Year 1999/2000 was £166,620.

2. Outputs

Patulin Detection

There was a project on the development of a rapid method for patulin detection in apple products using antibody techniques. Serious problems were found with the antibody, contamination and unsuitability and consequently the project was terminated. A 'follow-up' project to address some of these problems has now been re-considered. It is hoped that it will be able to start later this year.

Patulin Production

A project further investigating the formation of patulin (a type of mycotoxin mainly found in apple/apple products) in apples stored for juice production commenced in April 1997. Among the key findings of this completed project was that patulin production in apples did not appear to be related to the mineral status (and assumed firmness) of the fruit. The project concluded that the major risk factors for patulin formation in stored apples were:

- The presence of a significant level of *Penicillium expansum*.
- The bruising of fruit before controlled atmosphere storage, and bruising after storage if followed by an ambient storage period exceeding a few days.
- The ambient storage of fruit following withdrawal from controlled atmosphere storage.
- The inadequate grading of the fruits i.e. allowing some rotten fruits with high patulin levels to be passed for juicing.

A project looking at the possibility of reducing patulin in apple juice through controlling the horticultural practices in orchards begun in June 1998. Samples of bark, dead foliage, epiphytes from branches, soil, buds, leaf and fruit surfaces were taken from an orchard where pesticides have not been applied for many years. Sixty-five different *Penicillium* species were detected mostly from the outer bark.

As part of this project a method to reduce the overall patulin detection time using the gene probe was developed. This method was used to detect patulin in samples of bark, soil and twigs from the above orchard. The discovery of patulin in these samples supports the hypothesis that either the gene lives endophytically within apple trees or the trees can possess the patulin gene without the fungus. This may explain why patulin can be found in apple juice despite using undamaged samples.

In December 1998, forty-five stores containing Bramley apples from thirty-five orchards were sampled. By the end of May 1999, twenty-four samples had been analysed and none of them exceeded the EU limits.

In the year 1999/2000, ten orchards were selected for detailed examination, from the previous thirty-five orchards sampled in 1998/1999. Samples of soil, debris, leaves, bark and fruit were obtained in September 1999 and subjected to laboratory analysis to detect the presence of *Penicillium* species. Further samples of blossoms were obtained in May 2000.

Fruit samples from the 1999 harvest were placed in stores and sent for patulin detection. Agar plates were also exposed in stores and packhouses to check for the presence of *Penicillium* species in the atmosphere.

Twenty species of *Penicillium* were identified and two of these were found to contain the patulin gene in a non-functional form.

The analysis of fruit for patulin is still ongoing and so far, no samples have exceeded the EU limit.

Mycotoxins in grains

A project researching the effect of low and high input farming systems on the occurrence of mycotoxins in grains was started in April 1999. The cereals grown were observed to be free from serious mould infection hence, less fungicide than anticipated was applied to the sites for high input farming. As a result, no significant difference was observed between the low and high input farming systems. No definite conclusions could be drawn this year although the studies to date have been valuable in testing out procedures and allowing initial problems to be identified.

3. Dissemination Activity

The project covering the effects of horticultural practices in orchards, on reducing patulin contamination was presented at an international symposium on 'Biological Control Agents in Crop and Animal Protection'. A progress report on the project was also presented at a meeting of apple juice producers, at Brogdale, Kent.

4. Future Activity

There are no immediate requirements for research until the results of the existing contracts are available and have been analysed, whereupon further research will be required.

5. Comments

The Programme Advisor was Dr David Lindsay. However, in the future, due to the small size of the programme and the expertise within the Branch, this external advice is no longer required. The *Ad Hoc* Group on Mycotoxins will meet in the future to consider where this programme might be developed. The Group last met in Spring 1999 and identified no research requirements at that time. The surveillance programme for mycotoxins was identified as the important area to expand.

FS20b (now C03) – Reduction of mycotoxins in foodstuffs and animal feeds

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS2063 (C03001)	Further investigations on patulin formation in apples stored for juice production	Reading Scientific Services Limited	01/04/97	31/12/99	49,674
FS2089 (C03005)	Reducing patulin in apple juice through manipulation of horticultural practices in orchards	ADAS Consulting Ltd	01/06/98	31/05/01	72,013
FS2090 (C03002)	The effect of low and high input farming systems on the occurrence of mycotoxins in grain	Central Science Laboratory, York	01/04/99	30/09/02	21,055
FS2090 (C03004)	The effect of low and high input farming systems on the occurrence of mycotoxins in grain	KAS Mycotoxins	01/04/99	30/09/02	8,185
FS2090 (C03003)	The effect of low and high input farming systems on the occurrence of mycotoxins in grain	ADAS Consulting Ltd	01/04/99	30/09/02	15,693
TOTAL					166,620

FS20c (now T03) – Whole food approach to the assessment of risk from natural toxicants

1. Background

This programme consists of five projects and covers some diverse areas of interest in the field of potential health effects of natural constituents of foods. All of these projects have now come to an end.

The total spend on the programme in 1999–2000 was approx. £77k.

2. Outputs

The emphasis of the majority of the projects in this programme has been to characterise and evaluate the mechanisms of action of naturally occurring food chemicals that are thought to confer health benefits at dietary levels of intake.

Work at the Rowett Research Institute (T03002 formerly FS2055) has been characterising lignols, which are complex phenolic compounds present in plant foods. The interest in these compounds arises from the fact that like the phytoestrogens their metabolites can show estrogenic properties in *in-vitro* systems. It involved the development of suitable analytical methods for the characterisation of lignols in cell walls from a total of 15 fruits and vegetables and nine cereals for comparative purposes. A final report of the work is awaited.

Work at the MRC Toxicology Unit (T03004 formerly FS2074) has been investigating whether increased intake of vegetables and teas modulate the effect of high meat diets on colon cancer risk. The exfoliated colonic epithelial cells of human subjects maintained on controlled high red meat diets or high red meat and vegetable diets have been collected. These cells have been examined for altered phase II metabolism, the levels of enzymes involved in cell proliferation and for mutations in the K-ras gene that is frequently altered in colon cancer. A final report on this work is under review.

The effects of natural constituents in food on the processes responsible for cellular proliferation or apoptosis were investigated in a further two projects. Work at the Beatson Institute for Cancer Research (T03001 formerly FS2052) has demonstrated that flavonoids present in the diet can inhibit the cell division in normal, pre-neoplastic and neoplastic buccal cells. The project is also studying these effects on tumour inhibition *in vivo* using the Erb B2 mammary hyperplasia transgenic mouse model where the Erb B2 oncogene is overexpressed. This model provides an analogy to the pathology and metastatic potential of early human breast lesions. A final report on this work is awaited.

Work at the University of Essex (T03003 formerly FS2056) is shedding light on the mechanism of action of dietary isothiocyanates which seem to act specifically at the cell membrane leading to the increased expression of signalling factors that result in apoptosis. At present the mechanism appears to occur in part by an alteration in the redox potential of target cells that result from formation of a glutathione-isothiocyanate adducts. This leads to an efflux of the glutathione adducts from the cell and a decrease in cellular glutathione levels. However, other redox enzymes are likely to be affected since isothiocyanates activate the AP-1 and NF- κ B transcription factors, which are activated by the JNK pathway of apoptosis. It is known that alterations in the redox potential of the cell can lead to activation of genes leading to apoptosis rather than cellular proliferation. A final report on this work is under review.

The application of Bayesian probabilistic models to the development of objective methods for hazard assessments of risks posed by natural toxicants in the food chain have been investigated in a pilot project (FS2091). The analysis was carried out using data on the potato glycoalkaloid, solanine with emphasis on modelling human exposures and bioavailabilities. The project demonstrated the potential of these methods to enable objective conclusions to be made about the quantitative risks to specific sectors of the UK population and the uncertainties associated with these estimates. The preliminary analysis concluded that the UK potato supply posed no appreciable risks from solanine taking into account all of the known uncertainties in data. A final report is under review.

3. Dissemination Activity

A workshop was recently held jointly between the BBSRC and the Food Standards Agency to discuss the potential application of DNA microarray and related technologies to the evaluation of chemical food safety. Particular emphasis was given to the issue of assessing the safety of natural constituents of the food supply. Future work in this area may be encompassed within other Agency research programmes.

4. Future Activity

Policy issues that need to be addressed in this area of work are presently covered by the mycotoxins and phytoestrogen programmes. The need to consider new approaches for assessing the health risk-benefit of natural constituents of foods remains a priority. The potential for developing a European shared cost project to address some of these issues is being considered at the present time.

5. Comments

The Programme Advisor is Dr David Lindsay.

FS20c (now T03) – Whole food approach to the assessment of risk from natural toxicants

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS2052 (T03001)	The effects of dietary polyphenols on the proliferation and progression of human and mouse pre-malignant lesions	Beatson Institute for Cancer Research	01/10/95	30/06/99	16,000
FS2055 (T03002)	Distribution, structure and some effects on xeno-biotic metabolising enzymes of lignols found in fruit and vegetables	Rowett Research Institute	01/09/96	31/08/99	8,696
FS2056 (T03003)	Formation of alkylisothiocyanates from dietary glucosinolates. Effects on gene expression, cell growth and viability	University of Essex	01/09/96	11/05/00	22,846
FS2074 (T03004)	<i>In vivo</i> effects of vegetables and tea on human colonic bio-markers indicative of cancer prevention	Leicester University, MRC Unit	01/06/97	31/05/99	16,537
FS2091 (N/A)	Bayesian assessment of uncertainties in assessing natural chemical risks	IFR, Institute of Food Research	01/10/98	30/06/99	12,852
TOTAL					76,931

FS21 (now C01) – Chemical contaminants from food production

1. Background

Food may be contaminated from natural sources and from added fertilisers, including wastes, and supplements used in production or subsequent processing and from atmospheric pollution resulting in deposits on vegetation. The programme aims to develop an understanding of the mechanisms by which contaminants may enter the food chain, the relative importance of the different routes by which contamination may occur and the possible consequences for human health. Major activities include development of systems to predict the amounts of contaminants which may be present in the food chain in given circumstances, the ways in which these can be minimised or eliminated and new or improved methods for

detection of contaminants in food. The primary aim is to protect the consumer by creating a detailed understanding of any risks from inorganic and organic contaminants in food and ways in which these can be reduced or eliminated. The Agency may also use the results to influence international legislation.

The programme was initiated in 1990 and reviewed in 1994 and 1999. As a result of the earlier review the programme has recently concentrated on the behaviour of persistent organic pollutants (dioxins, polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs)) and the inorganic pollutants cadmium and arsenic in the food chain, although investigations have included a wider range of contaminants. The results of the 1999 review are addressed below.

The cost of this programme in Financial Year 1999/2000 was *ca* £755k.

2. Outputs

Accumulation of contaminants – Prediction and modelling

Investigations into the major factors which control accumulation of contaminants have been a consistent feature of this programme. A project to investigate the importance of vapours and particles in the uptake of persistent organic pollutants has shown that this is a significant route of uptake for these pollutants into vegetation. Vapour phase accumulation predominates for the more volatile chemicals in these series (which are also lost from vegetation more readily) with particle deposition as the dominant route for accumulation of the less volatile components.

When rivers flood sediments may be deposited on farm land. These sediments are a possible source of dioxins in the dairy food chain in some areas. During the winter of 1998/9 representative sites were sampled from the flood plains of rivers known to be contaminated. The dioxin contents of vegetation and animal products from these are compared with sites in the same vicinity but above the flood level.

Prediction of the extent to which dietary pollutants may be absorbed into the body when eaten are especially difficult to achieve. Two (linked) projects attempt to estimate the extent to which dioxins, PCBs and PAHs are absorbed from the human diet and the rate of excretion of absorbed material. The approach is to define foods with relatively high or low concentrations of the compounds of interest to prepare diets which are enriched or depleted compared to the average. By alternating these diets and measuring input and excretion during the changes it may be possible to estimate the rates of absorption and excretion of individual compounds. As expected, analyses show that vegan diets are low in dioxin content compared with those rich in animal fat, but PCBs may be higher in the

vegan diet. PAHs are intermediate with the higher molecular weight (and thus less volatile) species relatively low in vegetables, compared with the lower molecular weight species. These results are consistent with predictions from the air-herbage transfer experiments. Analysis of excretion products is in progress.

Another way to predict human exposure from the diet is to identify “markers” in the urine which may indicate the amount of specific compounds absorbed. A pilot project showed that this approach was feasible for the phthalates (a group of chemicals widely used in plastics, including those used in food containers). A larger study involved about 300 volunteers. This group recorded details of all food consumed over a four week period and collected their urine for part of this time. Analysis of urine samples will identify those individuals with high concentrations of “markers” This will then be correlated with foods consumed. Sample collection is complete but analysis of urine has been delayed by the relocation of the Central Science Laboratory.

Urinary markers are also used in a project which investigates whether there are adverse effects of nitrate at intake levels commonly encountered in winter in some vegetables. Volunteers consume low nitrate meals for a day followed by a meal which includes high nitrate vegetables. Blood, saliva and urine samples are tested for markers of effect. Preliminary results show that one marker, nitro-tyrosine, is increased in the urine after a nitrate rich meal but no modified proteins are seen in the blood. The work will be extended to a larger group of volunteers and determine whether the nitro-tyrosine comes from breakdown of modified proteins or from a detoxification reaction in the stomach.

Investigations into contaminants transfer in the food chain have resulted in a number of predictive mathematical models. Most of these are applicable to a limited range of circumstances and/or chemicals reflecting the data on which they were based. A project to compare the models and if possible integrate them into a user friendly system is in progress. This has proved more complicated than originally proposed. The study will also identify which areas of uncertainty have most influence on the models, aiding selection of future research targets.

New methods of analysis

Two projects which commenced during the year aim to provide rapid screening methods for a wide range of contaminants. One of these will characterise and validate several novel systems for a range of contaminants. It may also be possible to automate the application of these systems. The second will examine the potential of a new development in mass spectroscopy for rapid and specific assessment of a range of contaminants in food.

An earlier pilot study demonstrated the feasibility of a system for rapid extraction of PCBs from food. Further development of this system is in progress but initial results suggest it may be inefficient at low concentrations, limiting its value for routine analyses.

It is sometimes useful to assess a functional property common to a range of chemical structures. An example is hormonal activity (endocrine disrupters). This is particularly challenging in a complex matrix such as food. An *in vitro* system which uses a genetically modified yeast expressing the human oestrogen receptor, is being investigated as a possible assay system. The test has been shown to detect a range of chemicals with oestrogen-like activity, but there is an inconsistent response to some which require metabolic activation. Work in progress aims to overcome this limitation.

Methods to control transfer of contaminants

Two projects to reduce the uptake of contaminants by plants are nearing completion. The first examined addition of various minerals to contaminated soils as a way to reduce the uptake of cadmium and mercury. Some of these treatments were successful in field trials during 1999. A second round of trials is in progress to see whether the results can be repeated for a second season with a different range of crops. The second project evaluates simple treatments such as composting as a way to reduce transfer of organic chemicals from sewage sludge to crop plants. Trials with pot-grown plants in a greenhouse showed that the treatments are potentially useful and a field trial is in progress.

In order to control contamination it is usually necessary to determine the source. Some milk samples and infant formulae have been found to contain more iodine than predicted. Although iodine is an essential nutrient, excess can have unwanted side effects and a project is attempting to identify the chemical form of the iodine present as an aid to determining the source. This should allow the concentration to be controlled more closely.

The technique of Hazard Analysis by Critical Control Points (HACCP) is well established as useful for the control of microbiological contamination of food. A desk project examined the possibility of extending this to the control of chemical contaminants. The report identifies major points for control of contaminants and may be of benefit to many food producers.

3. Dissemination Activity

Results from this project have been published in internationally recognised journals and presented at national and international meetings and conferences. At least 15 papers have been published or are in press over

the last year. Although publication is actively encouraged, there is a trend for this to lag behind project reports, sometimes by a significant margin.

The programme review coincided with a contractor's meeting, at which current projects were presented. This was held in Brighton in December.

4. Future Activity

A project to assess the persistence of alkyl phenols in soils and the potential for uptake into food crops will commence during the year. A further project will compare levels of lead and manganese in soils adjacent to a major highway with more remote locations. This will establish a baseline so that the effect of discontinuing leaded petrol can be assessed. Concentrations of the platinum group metals (from catalytic converters) will also be measured in roadside vegetation.

Development of an assay system which separately identifies the isomers of the alkyl phenol ethoxylates (a range of detergents, some of which are potential endocrine disrupters), delayed by the relocation of CSL, will now begin. Separate identification is useful because not all of the isomers are biologically active.

Improvements in analytical methodology will continue to be investigated where appropriate. These will concentrate on chemicals from the current priority list including those of recent concern.

Following the programme review and transfer of the programme from MAFF to the Food Standards Agency, there will be an increasing focus on physiological relevance of exposures while work on land remediation will be phased out.

5. Comments

The programme was reviewed in December.

The main conclusion from the review were:

- The programme is still relevant, but must continue to respond to changing priorities and practice.
- Predictive models should be kept to “state of the art” by judicious investment in improvements
- Bioavailability studies should be directed to protection of public health, including investigating the physiological relevance of exposure where appropriate

- There should be fewer but larger projects, possibly with international co-operation
- Analytical method development should remain a priority
- Dissemination activities should be actively encouraged

The programme advisor is Dr G H Pigott.

FS21 (now C01) – Chemical contaminants from food production

Project Code	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS2176 (N/A)	Organic contaminants in sewage sludge amended soils, further studies of their environmental and food safety significance	Lancaster University	01/03/96	28/02/99	13,851
FS2190 (C01021)	The use of sorptive minerals to minimise the uptake of arsenic (As) and cadmium (Cd) by food crops in contaminated	Reading University	01/07/96	30/06/99	32,966
FS2191 (C01022)	Development of a mechanistic understanding and model of the air-herbage transfer of persistent organic contaminants	Lancaster University	01/02/98	31/01/01	133,307
FS21112 (C01001)	Simple methods to reduce potential transfer of organic chemical residues from sewage sludge amended soils to food crop	UL, Wye College	01/10/97	30/09/00	67,632
FS21119 (C01003)	Assessment of total oestrogenic environmental contaminants in food with recombinant human receptor systems	Veterinary Laboratories Agency	01/04/98	31/03/01	69,867
FS21120 (C01004)	Food hazard analysis by critical control points (HACCP): Development & evaluation of a whole food chain approach	UL, Wye College	01/11/98	31/10/99	41,213

Project Code	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS21121 (C01005)	Development of analytical methodology and measurement of dietary exposure to alkylphenols and alkylphenol ethoxylates	Central Science Laboratory, York	01/06/98	31/05/99	9,335
FS21122 (C01006)	The effects of processing on lead and cadmium levels in food	AEA Technology	01/05/98	30/04/00	41,060
FS21123 (C01007)	Study of the effects of PCDD/Fs & PCBs in river sediment, deposited on pasture by flooding, on concs. in cows' milk	Central Science Laboratory, York	01/08/98	31/03/00	83,294
FS21125 (C01008)	Rapid, single step PCB extraction from liquid milk using perfluorocarbon fluids	Chimaeron Ltd	01/09/98	30/04/00	26,603
FS21125 (C01009)	Rapid, single step PCB extraction from liquid milk using perfluorocarbon fluids	Central Science Laboratory, York	01/09/98	30/04/00	12,640
FS21127 (C01010)	Measuring the bioavailability of human dietary intake of dioxin-like compounds	Birmingham University	01/10/98	30/09/00	77,505
FS21128 (C01011)	A review of the current suite of models used by MAFF's FCD & RSND	Westlakes Research (Trading) Ltd	01/10/98	31/03/00	46,800
FS21129 (C01012)	Measuring the bioavailability of human dietary intake of PAHs, phthalates and aromatic hydrocarbons	Birmingham University	01/10/99	31/10/01	18,719
FS21130 (C01013)	Dietary nitrate consumption: an investigation of biomarkers of DNA & protein damage in humans	United Medical and Dental Schools, Free Radical Research	01/09/99	30/08/02	40,240
FS21131 (C01014)	Rapid & automated methods for the screening of foods for the presence of a wide range of organic contaminants	Leatherhead Food Research Association	01/07/99	31/12/00	20,091

Project Code	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS21132 (C01015)	Feasibility study of rapid detection of some food contaminants using MALDITOF MS	Central Science Laboratory, York	01/10/99	30/09/01	2,954
FS21133 (C01016)	Investigation of iodine species in milk and infant formulae	Central Science Laboratory, York	01/09/99	31/08/00	13,279
FS21134 (C01017)	Development of routine methods for the determination of total and organometallic mercury in food	Plymouth University	01/12/99	30/11/00	3,963
TOTAL					755,319

FS22 (now A03) – Chemical contaminants from food contact materials and articles

1. Background

The purpose of this programme was to provide a sound scientific basis for UK input to international negotiations in the European Union (EU) and at the Council of Europe. Research is also carried out in this programme to improve understanding of chemical migration from materials and articles in contact with foodstuffs to ensure that substances from this source do not endanger health.

This programme includes the quantification of chemical migration and the development of appropriate test protocols, as well as more fundamental work to increase understanding of the factors affecting chemical migration.

The cost for this programme in Financial Year 1999/2000 was £580k.

2. Outputs

There is an increasing range of different types of material researched in this programme. There is new work on glazed ceramic ware, cork and synthetic stoppers, and unusual and non-traditional types of wood. Work continues on inks and chemicals in coatings and on paper and board. There is also work to identify the stages in producing paper and board where contamination might occur. Work is nearing completion on food contact rubber and elastomers, including the effects of ageing and sanitation procedures on chemical migration from these materials.

Work continued to support UK negotiations on plastics in contact with food. The use of overall migration testing for plastics has been studied. Using this approach to test for specific migration was found to be applicable for polymers with a low intrinsic migration. Related work developed a protocol but this would require additional clean-up to detect low levels of chemical migrants. A highly topical project is investigating the possibility of taking account of food consumption factors as well as packaging-use factors within the EU regime for controlling chemical migration from food contact plastics. A polymer-specific mass spectroscopic library is being developed. This will contain potential migrants from food-grade plastics.

There are several studies to improve understanding of chemical migration. These include investigations of the effects of low temperature storage and the freeze thaw cycle. There is also a project to investigate and certify a reference material for fatty contact between packaging and food. Work also continues on a study of five model substances to develop and validate a predictive approach to assessing risk to consumers of exposure to chemical migration from food contact materials.

Methodological work has continued. A project aimed at developing an immunoassay screening test for BADGE and related migrants from food contact plastics was completed and reported. There has also been work on migration testing for electroplated and dipped metal-ware. A test protocol has been devised on functional barriers, based on generic criteria. A definitive test for set-off transfer of pigments and other non-volatile substances between packaging surfaces is being developed. It is intended to produce a method of identifying invisible transfer of chemicals to flexible food packaging.

3. Dissemination Activity

The programme was reviewed in 1999 and 2000 by the Working Party on Chemical Contaminants from Food Contacts Materials and Articles. Reports on these meetings have been distributed to a wide range of interested parties both in the UK and overseas. They are available together with an Explanatory Note on the research programme from FCM Unit, Food Standards Agency, Room 216, P.O. Box 31037, London SW1P 3WG, Tel. No. +44 (0)20 7238 6528, fax +44 (0)20 7238 6124.

A panel of consultees reviewed the programme in October 1999 and concluded that it was well managed and relevant, and provided a significant contribution to UK policy and legislation. Food Safety Information Bulletins announced the availability of eight final project reports in 1999/2000. Four journal papers reporting the results of the research programme on food contact materials were published.

4. Future Activity

Major new projects will start in Financial Year 2000/01. An area of work new to this programme will be the investigation of elemental migration from glass in contact with food. This study will provide a review of the factors relevant to elemental migration from glass. Another project will investigate the migration of metals and coating materials from kitchenware products. A third new project will investigate the occurrence of chemical migration from secondary packaging and subsequently into the food.

5. Comments

The Programme Manager is Dr David Watson of the Food Standards Agency's Chemical Safety and Toxicology Division.

FS22 (now A03) – Chemical contaminants from food contact materials

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS2223 (A03002)	Chemical composition and migration levels of packaging adhesives.	De Montfort University	01/06/95	31/03/00	38,269
FS2238 (N/A)	Development of a strategy for the effective enforcement of food contact plastics legislation.	RAPRA Technology	01/06/96	30/04/99	6,622
FS2239 (A03009)	Methods and searchable spectroscopic libraries to identify substances migrating above a 1ppb threshold	Central Science Laboratory, York	01/04/97	31/03/00	82,798
FS2240 (A03010/11/12)	Definitive test for set-off of pigments and other non-volatile substances for flexible packaging	Laser Installations	01/06/97	31/05/00	115,347
FS2242 (A03015)	Immunoassay screen for contaminants from food contact plastics	Laboratory of the Government Chemist	01/04/97	31/06/00	965
FS2244 (N/A)	Overall migration as a measure of both the safety and quality of plastics used in connection with food and drink	RAPRA Technology	01/04/97	31/04/99	10,346

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS2245 (A03016)	Investigation of the migration of metals from glazed ceramic ware	Central Science Laboratory, York	01/04/98	31/07/00	44,560
FS2246 (A03017)	A flexible food consumption approach using food type factors and packaging usage factors leading to refined restrictions on migration	Central Science Laboratory, York	01/04/98	30/09/99	8,766
FS2247 (A03018)	Investigation into the effects of the freeze thaw cycle on chemical migration from packaging foods	Central Science Laboratory, York	01/04/98	30/09/99	5,234
FS2248 (A03019)	Further research on chemical migration from food contact rubber and other elastomers	RAPRA Technology	01/04/98	31/06/00	74,820
FS2249 (A03020)	Investigation of the migration of chemicals from agglomerate and natural cork stoppers	Central Science Laboratory, York	01/04/98	30/09/00	29,293
FS2250 (A03021)	Migration from recycled paper and board to dry foods. Research into the factors involved, leading to practical avoidance and amelioration measures	Central Science Laboratory, York	01/10/99	31/03/02	69,604
FS2251 (A03022)	A systematic investigation into chemical migration from inks and associated coatings used on the food contact surface of packages	Central Science Laboratory, York	01/10/99	31/12/01	37,370
FS2252 (A03023)	Strategy for assessing risk and assigning priorities to chemicals used to make food contact materials. A tiered approach with progressive refinement to calculate exposure levels	Central Science Laboratory, York	01/10/99	30/09/01	13,972

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS2253 (A03024/25)	Collation and review of information on the use of unusual and non-traditional types of wood used as food contact materials	Leatherhead Food Research Association	01/08/99	31/01/01	18,093
FS2254 (A03026)	Investigation of the migration of inorganic contaminants into dry food from packaging made from recycled paper and board	Imperial College	01/06/99	30/09/00	24,037
TOTAL					580,096

FS29 (now E01) – Improved methods of analysis

1. Background

The objective of this programme is to ensure that analysts have available methods of analysis which are fully validated, or where a method of analysis is required but not available, to develop such methods. The programme also embraces aspects of quality assurance for sampling/analysis, e.g. the operation of proficiency testing schemes so that the analytical performance of laboratories can be assessed and internal quality control requirements.

There are compositional criteria (including for additives and contaminants) specified in legislation. Such criteria may be either directly specified in individual Regulations or be specified by reference to the general provisions of the Food Safety Act. For legislative purposes methods of analysis must be validated to recognised international standards. In addition laboratories must not only use validated methods of analysis but must also be confident that they are doing so proficiently; this may be best assessed by participation in proficiency testing schemes. Such considerations also apply to surveillance exercises where the results obtained must be fit-for-purpose. This programme provides information on how this may be achieved.

The programme consisted of discrete areas of work, dealing with;

- the development and validation of specific methods of analysis,
- the investigation into specific methodology problems for food analysts, and

- the estimation of the measurement uncertainty of a result with reference not only to the analytical uncertainty but also the sampling uncertainty.

The cost of the programme in the 1999/00 financial year was ca. £258k at FEC rates.

2. Outputs

The programme has achieved the following:

Fitness for Purpose of Food Analysis and Sampling

"Fitness for Purpose (FFP) is the property of data produced by a measurement process that enables a user of the data to make technically correct decisions for a stated purpose (Thompson and Fearn 1995)". As more and more resources are applied to analysis in terms of instrumentation to achieve lower detection limits, improved precision and accuracy through rigorous quality assurance, little effort has been spent on assessing whether the methods and sampling protocols are fit for purpose. An initial project applied FFP theories to historic costing and analytical data obtained from specific examples of food analysis and sampling. Use was made of Genstat software to calculate contours of cost as a function of targeted concentration and the standard deviation of sampling and analysis.

This work has been extended to more practical applications in the food sector. This is showing that it is necessary possible to combine considerations of both sampling and analysis but that in many instances it will be difficult to achieve this in practice as there will be a reluctance to undertake multiple sampling and analysis procedures.

Development of A Protocol Handbook for the Application of Capillary Electrophoresis to Food Analysis

Capillary electrophoresis (CE) has several advantages over liquid chromatography with respect to the relative ease of sample preparation and high resolving power. As yet, the practical application of CE to food analysis has been limited. This is attributed to the scarcity of appropriate methods, method development and optimisation being hampered by a lack of practical knowledge of CE in food analysis laboratories, including those of control laboratories.

The objective of the project is to meet the urgent need for a widely applicable protocol handbook for the application of CE to food analysis. This has been finalised and is published.

Quality Assurance Procedures and Laboratory Performance in Microbiological Laboratories

An investigation has previously been carried out to investigate whether there is any relationship between a laboratory's analytical performance, as measured by results from participation in proficiency testing scheme, and the internal quality control measures adopted by the laboratory. It was also noted whether the laboratory was third-party accredited or not. The first study on chemical laboratories found that there was no correlation between laboratory performance and accreditation status, but that there is a good correlation between the extent of internal quality control procedures used and laboratory performance as measured by proficiency test results. An internationally accepted protocol was drawn up and published as a result of this work.

The same exercise has been repeated for microbiology laboratories. Here the situation is not so positive, and no definitive correlation can be made. In contrast to the situation with the chemical laboratories, most laboratories in the survey are now accredited, and this may also have influenced the conclusions which were able to be drawn from the project.

3. Dissemination Activity

Results from the projects in the Methods of Analysis and Quality Assurance Programme have been published in internationally recognised scientific journals and published in national and international meetings and conferences.

4. Future Activity

The following have been shown to be needed to be addressed:

- The clear general need for newly developed or modified sampling protocols to have associated with them estimates of sampling uncertainty has been identified and should be implemented. This will allow end-users of analytical data to estimate the combined uncertainty of the results they are studying, an essential prerequisite to interpreting the data correctly. In the first instance, R&D support is required for producing a few such new sampling protocols complete with uncertainty information, to act as examples for future developers of sampling protocols. The examples while typical should be as diverse as possible so that difficulties associated with particular materials can be assessed.
- With regard to fitness-for-purpose there is a clear need to extend the project into as many real scenarios as possible

to obtain reliable estimates of the “cost of getting it wrong”. This is of particular interest to the FSA now that their surveillance exercises are open to greater scrutiny and also to the wider industry where financial considerations are paramount.

There are a number of current projects ongoing in the programme, the results of which will be reported during 2000/01. These included:

- The development & validation of a method for the determination of ethyl carbamate in wine.
- A replacement for the alpha amylase test to determine the heat treatment of liquid whole egg.
- The assessment of various homogeneity assessment procedures for proficiency testing test materials.
- The determination of organic acids in egg products using capillary electrophoresis.
- The preservation of sub-samples obtained under Regulations made under the Food Safety Act, and which may be used in dispute situations.

5. Comments

The objectives of the programme, to ensure that such data may be accepted with confidence, is one that under-pins all legislative and surveillance activities undertaken both within the UK and on behalf of the Food Standards Agency specifically. The programme provides direct information on how this may be achieved in certain areas as well as providing general information of importance to those making judgements on data quality.

The Programme Manager in 1999/00 was Dr Roger Wood.

FS29 (now E01) – Improved methods of analysis

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS2917 (E01016)	Modern statistical methods in analytical chemistry	Royal Society of Chemistry	01/09/97	31/08/99	9,387
FS2921 (E01021)	Fitness for purpose of food analysis	Central Science Laboratory, York	01/05/98	31/01/00	24,000
FS2923 (E01023)	Quality assurance procedures and microbiology laboratory performances	Campden & Chorleywood Food Research Association	01/10/98	30/09/99	23,846
FS2924 (E01024)	Validation of a method for the detection of melange in pasteurised liquid egg	Central Science Laboratory, York	01/08/98	31/07/99	19,317
FS2925 (E01025)	The development & validation of a method for the determination of ethyl carbamate in wine	Central Science Laboratory, York	01/08/98	30/09/99	26,716
FS2926 (E01026)	Development of a protocol handbook for the application of capillary electrophoresis to food analysis	Reading University	01/11/98	31/10/99	51,813
FS2927 (E01027)	A replacement for the alpha amylase test to determine the heat treatment of liquid whole egg	Central Science Laboratory, York	01/08/98	31/07/00	64,000
FS2931 (E01031)	Guidelines for the preservation of official samples for analysis	Campden & Chorleywood Food Research Association	01/06/99	30/11/00	18,038
FS2934 (E01034)	Optimized uncertainty at minimum cost to achieve fitness-for-purpose in food analysis	Sussex University	01/10/99	30/09/02	21,067
TOTAL					258,184

FS30 (now T07) – Food intolerance

1. Background

Food allergy and food intolerance are important, continuing and, in the case of food allergy, possibly increasing health issues. A food intolerance research programme was established in 1994, the main aims of which have been to identify the causes and mechanisms of food allergy and intolerance and to characterise the factors that influence the pathogenesis of these diseases. It is anticipated that collectively the research projects commissioned and supported by this Programme will provide information of value in the identification of steps to reduce the incidence and severity of food allergy and intolerance. In recent years specific objectives have included: the determination of the extent to which hyperactivity and other behavioural problems can be traced to foods and food ingredients; development of the approaches to the design and construction of databases of allergenic epitopes expressed by proteins; the development of methods for the identification and characterisation of food allergens; and examination of whether there exists any scientific or clinical basis for the apparent differences in the allergic sensitivity and reactivity of young children and adults to peanuts and tree nuts.

Future objectives are:

- to determine whether and to what extent the prevalence of food allergy is increasing;
- to examine the influences of age and route of exposure on the development of food allergy;
- to consider what approaches may be available to identify individuals who are at risk of developing allergic sensitisation to foods;
- to characterise the structural basis for allergenicity and the properties that confer on proteins the ability to induce allergic sensitisation;
- to develop further suitable methods for the identification and characterisation of food allergens and;
- to determine whether there are individuals with Attention Deficit-Hyperactivity Disorder who might benefit from dietary intervention.

The cost of this Programme during the financial year 1999–2000 was ca. £967k.

2. Outputs

1. **Identification and characterisation of food allergens**

The focus of attention here has been on the investigation of several approaches to the identification of potential food allergens and of proteins associated with the oral allergy syndrome. Among the methods examined have been a Brown Norway rat model in which test proteins are administered by intraperitoneal injection with adjuvant or by gavage dosing of adjuvant treated animals. A second method is based on the use of basophil cell lines which permit measurement *in vitro* of allergen-specific IgE antibody. The merits of these approaches are continuing to be evaluated.

2. **Cross-reactivity in peanut allergy**

The primary objective here is to examine the relationship between allergic sensitisation to peanuts and the development of cross-reactivity to other food allergens. The approach hopes to distinguish between true cross-reactivity and parallel sensitisation. In a separate series of investigations the aim is to determine whether and to what extent cross-reactivities in peanut and tree nut allergies increase with age.

3. **Development of allergen databases**

Progress has been made in developing databases that will provide access to listings of known allergenic epitopes and their amino acid sequences, linked to information on the type of allergic disease with which they are associated.

4. **Mechanisms and prevalence of food allergy**

Among the issues being addressed here are: (1) the influence of maternal diet and *in utero* exposure to allergen on the development of allergic sensitisation to foods, (2) the influence of infant feeding practices on the development of food allergy, (3) the roles played by the mucosal immune system in the induction of allergic responses to cows' milk and (4) the impact of exposure and sensitisation to storage mites on food allergy and intolerance.

5. **Hyperactivity**

A study is in progress to determine the influence of artificial food colourings and a preservative on the behaviour of children, and to examine whether atopy or hyperactivity increases vulnerability to such effects.

3. Dissemination Activity

Among the publications deriving from the investigations summarised above are the following: Meredith C & Atkinson HAC (2000) Development of models to predict the allergenic potential of food proteins. *Toxicological Sciences* **54**, 144. Murch SH (2000) Immunologic tolerance and dietary antigens. *Paediatric Research* **47**, 430. Norris F, Williams C, Larkin M, Deacock S & Morgan JB (1998) Total IgE levels in atopic and non-atopic

preterm infants: effect of feeding practices. *Journal of Allergy and Clinical Immunology* **101**, 783. Norris FJ, Williams CM, Larkin MS, Morgan JB & Hampton SM (1999) Total IgE levels and allergic outcome in preterm infants. *Journal of Allergy and Clinical Immunology* **103**, 460. Opara M, Oehlschlager S et al (1999) An in vitro cell based model for assessing the potential of allergens to release mediators through the cross-linking of IgE. *Toxicology in Vitro* **13**, 811.

In addition papers have been presented, or are planned to be presented, at the following scientific conferences: American Gastroenterological Society, Orlando, 1999; European Society of Paediatric Gastroenterology, Hepatology and Nutrition, Warsaw, 1999; World Congress of Mucosal Immunology, Amsterdam, 1999; American Academy of Allergy, Asthma and Immunology, Orlando, 1999, American Academy of Allergy, Asthma and Immunology, San Diego, 2000; Society of Toxicology, Philadelphia, 2000; British Society for Immunology, Harrogate, 2000; International Congress of Allergology and Clinical Immunology, Sydney, 2000; World Congress of Paediatric Gastroenterology, Boston 2000.

FS30 (now T07) – Food intolerance

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS3006 (N/A)	The development of diagnostic assays for food induced anaphylaxis.	Southampton University	01/04/95	31/03/99	21,764
FS3007 (T07001)	The prevalence and natural history of peanut allergy and investigation into its genetic environmental and immunological	St Mary's Hospital Medical School	01/12/95	31/03/01	55,534
FS3009 (T07002)	Development of food intolerance in atopic and non atopic families: influence of maternal nutrition and infant feeding	Surrey University, Biological Sciences	01/10/96	31/03/00	132,446
FS3010 (T07003)	Investigation of the immunological mechanisms inducing cows' milk sensitive enteropathy	Royal Free Hospital School of Medicine	01/10/96	31/01/02	57,952
FS3012 (N/A)	Prevalence and pathogenesis of food allergies in children	Bristol University	01/04/96	31/03/99	25,363

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS3015 (T07014)	Do food additives cause hyperactivity & behaviour problems in a geographically defined population of 3 & 5 year olds?	David Hide Asthma & Allergy Research Centre	01/07/97	30/06/00	82,360
FS3017 (T07005)	The effect of exposure to food protein via maternal sources in the development of food allergy in infants with a family	Southampton University	01/05/97	30/04/01	153,201
FS3019 (T07006)	Adverse reactions to foods: Bibra international	BIBRA Toxicology International	01/09/97	31/08/00	21,350
FS3020 (T07007)	A clinical trial to investigate potential allergenic reaction from the ingestion of storage mites	University Hospital of South Manchester	14/07/97	13/07/99	32,031
FS3023 (T07008)	Development of methods to predict the allergenic potential of genetically modified foods ... (formerly FS0218)	BIBRA Toxicology International	01/05/97	31/03/00	121,222
FS3026 (Not yet assigned)	Development an in vitro screening method for allergens in novel foods	Central Science Laboratory, York	01/04/98	30/09/00	71,711
FS3027 (T07010)	Allergic cross-reactivity in peanut allergy	UL, UCL Medical School	01/06/99	31/05/02	52,573
FS3028 (T07011)	Immunochemical reactivity to peanuts & nuts in allergic individuals	IFR, Institute of Food Research	01/04/99	31/03/02	119,167
FS3029 (T07014)	Investigate cross reactivities toward peanut & other nuts in relation to the age of the allergic individual	Withington Hospital	02/08/99	01/08/00	5,955
FS3029 (T07012)	Investigate cross reactivities toward peanut & other nuts in relation to the age of the allergic individual	Central Manchester Healthcare NHS Trust	02/08/99	01/08/00	13,940
TOTAL					966,569

Food Safety (MINIM PP1:02)

FS10 (now B06) – Hygienic food processing

1. Background

The UK food industry continues to offer consumers a wide variety of traditional and newly developed food products, of high organoleptic quality and high assurance of microbiological safety. Consumers' perceptions of food processing and preservation, together with efforts by the industry to improve the nutritional status of foods, have resulted in milder processes and lower levels of many traditional preservatives. A primary aim of this programme was to ensure that those milder processes deliver microbiologically safe foods.

Despite improvements in agriculture and animal husbandry, it remains impossible to produce raw agricultural products that are always free from all the known pathogenic micro-organisms of concern to human health. The carriage of pathogens by animals has been reduced, and the hygienic production of foods that are eaten raw or with minimal cooking has improved immeasurably in recent years. This programme has focussed on further improving food safety by eliminating those low numbers of pathogenic micro-organisms that inevitably occur from time to time on some raw foods, and consequently enter the food processing environment. They must be eliminated by the applied process, or their survival and growth in the food processing environment prevented or minimised. The information gained facilitates implementation of HACCP and assists microbiological risk assessments.

The total spend on FS10 in FY99-00 was ca. £80k.

2. Outputs

Increased demand for unprocessed fruits and vegetables, together with the common use of very mild heat processes, drew attention to the ineffectiveness of decontamination practices used widely in the food industry – decontamination under reduced pressure using steam and organic acids was explored (FS1043, ended in 1999). Steam at ambient pressure proved to effectively reduce numbers of vegetative pathogenic bacteria and spoilage micro-organisms on a range of fruits and vegetables, with visible damage to the fruit and vegetables the main limiting factor.

Development of new “slippery surface” materials to prevent biofilm establishment during food manufacture and processing (FS1047, ended in 1999), demonstrated that grafting appropriate synthetic polymers to materials could significantly suppress bacterial adhesion. Patterns of adhesion differed with different bacteria tested, suggesting different mechanisms of adhesion. The project demonstrated the potential to modify surfaces in food processing environments to reduce greatly bacterial adhesion and subsequent of micro-organisms to foods.

Microbiological risk assessments from raw foods through harvesting and processing to consumption has identified the urgent need for an approach that takes account of as much available information as possible. A network approach was developed (FS1046, extended to 2000), initially for strains of *Clostridium botulinum* able to grow and produce toxin at refrigeration temperatures, but using rules and procedures that can then be applied to other microbiological hazards. Having demonstrated the principle, the Belief Network is being modified to contain a front-end interface for use by regulators, to take account of subjective information, and to incorporate decision nodes to support decision making.

3. Dissemination Activity

This programme was designed to run parallel with, and feed into, the LINK programme on Advanced and Hygienic Food Manufacturing.

A fifth joint workshop with LINK on Hygienic Food Processing for Engineers and Microbiologists was held on 11 January 2000 in London. Lectures included Localised cooling (AFM3), Detection of volatiles via fly antennae (Bridge LINK), Slippery surfaces to prevent biofilms (FS1047), Food structure and microbial growth (FPS123), Microbial inactivation during food processing (BBSRC), Risk assessment – a network approach (FS1046), Effective decontamination processes (FS1043, MAFF Fellowship, EU), Novel high oxygen and noble gas modified atmosphere packaging (FAIR Shared Cost CT96-1104). In addition posters represented projects in LINK, FS10, FS15, FS32 and Shared Cost CT97-3129.

Results from the programme are being disseminated in the scientific literature and by presentation at colloquia, workshops, national and international meetings.

4. Future Activity

This programme ends in 2000.

5. Comments

Feedback from delegates who attended the Fifth Workshop on Hygienic Food Processing shows continued and wide interest in all aspects of research to ensure food safety. The workshops have resulted in several new collaborations between different scientific disciplines.

The Programme Adviser is (Dr) Terry Roberts OBE, Food Safety Consultant.

FS10 (now B06) – Hygienic food processing

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS1046 (B06001)	Risk assessment for microbial contamination hazards: A network approach	IFR, Institute of Food Research	01/04/96	30/06/00	15,942
FS1047 (N/A)	The development of new slippery surface materials to prevent biofilm establishment during food manufacture	IFR, Institute of Food Research	01/06/96	31/05/99	42,084
FS1050 (B06002)	Evaluation of barriers to usage of food hygiene management systems throughout the UK food industry	University of Wales Institute, Cardiff	01/09/96	31/08/99	21,244
TOTAL					79,270

FS12 (B09) – Detection and separation of food pathogens

1. Background

Microbial methods used in the food industry for the detection of pathogens are of limited reliability and are relatively slow in producing results (some methods take several days). Reliability is significantly affected by the efficiency of detachment and separation of bacteria from the food matrix and the sensitivity and selectivity of the detection method in accurately signaling the presence of the target organism. Although reducing the time taken for results to be available is a desirable goal, it is the reliability of the results obtained which is of primary importance.

The overall objective of this Programme is to “improve methods for the detection of major food borne pathogens so that they can be used

- by the food industry to inform food hazard management systems,
- to support enforcement authorities and
- to facilitate the identification and tracing of organisms causing illness."

The objectives of the projects within the Programme are to:

- develop reliable methods for the detection and separation of microorganisms from food matrices,
- develop methods for concentrating the separated microorganisms in a system suitable for presentation to the detection method,
- develop selective and sensitive methods for the detection of low numbers of viable pathogenic bacteria, infective virus particles, infective parasites or harmful levels of microbial toxins in foods,
- ultimately achieve real time availability of reliable microbial information to support policy objectives of ensuring the safety of the nation's food supply.

This Programme began in 1996 and will finish in 2002. The cost for this Programme in Financial Year 1999/2000 was ca. £740k.

2. Outputs

The main achievements of the Programme during the past year are as follows.

FS1223 (B09001): A method for detecting *Cryptosporidium parvum* oocysts and *Giardia duodenalis* cysts in natural mineral waters.

Rapid, efficient, reproducible, user friendly methods for isolating, concentrating and identifying *Cryptosporidium* oocysts and *Giardia* cysts in natural mineral waters have been developed. The molecular methods developed appear robust, provide added significance to morphological detection and can be used when novel molecular typing schemes emerge. In addition, they provide significant information for industry, public health officials, epidemiologists and regulators. Methods have been developed both for small volumes (for public health and epidemiological tracing purposes) and for large volumes (quality assurance prior to and following exploitation) of mineral waters.

FS1242 (B09002): Rapid detection, quantification and molecular characterisation of thermophilic campylobacters in foodstuffs and related environments.

Two novel PCR assays (a TaqMan PCR assay and a PCR-ELISA assay) have been developed which allow the sensitivity and specific detection of campylobacters in foods using a user-friendly format. The TaqMan assay was found to be as sensitive as conventional culture methods but reduced the time taken for detection in enrichment broth samples to three hours. The TaqMan assay was found to have a sensitivity of 94%, a specificity of 93%, positive predictive value of 96% and a negative predictive value of 93% when compared with enrichment culture. The PCR-ELISA assay was demonstrated to be as sensitive and specific as conventional culture based methods. Application of the PCR-ELISA assay to 48 hour enrichment broth samples demonstrated the assay to have a sensitivity of 97%, a specificity of 95%, positive predictive value of 97% and a negative predictive value of 95%.

FS1245 (B09004): Sono-chemical signal development as a novel generic method for rapid, in situ and specific detection.

The performance of vapour and sonochemical detection have been optimised and evaluated to enable comparison of their potential in microbiological hazard detection, as a rapid screening method in the laboratory, near real time monitoring at or on line and for revealing potential hazards in packaged food.

FS1249 (N/A): Development of assays for Clostridium botulinum toxins in food.

The characterisation of mAbs to non-proteolytic type B toxin and optimisation and assessment of a non-proteolytic type B toxin assay have been completed.

FS1260 (B09006): Preparation of 'validation samples' for use in the evaluation of microbiological methods.

Investigations on the survival and recovery of *Salmonella* in laboratory-prepared cream cheese in the presence of a natural background flora have been completed. Standard laboratory production protocols have been produced for the manufacture of 'naturally contaminated' cream cheese and mayonnaise which can be used by other laboratories for the production of 'validation' samples for the assessment of new or existing microbiological methods. These standard production protocols have been verified using microbiologists in another laboratory.

FS1264 (B09007): Develop and validate methods to detect and characterise verocytotoxin producing Escherichia coli in foods.

Enrichment medium and protocols have been developed and optimised to detect and characterise verocytotoxin producing *Escherichia coli* in foods.

FS1265 (B09008): Accelerated detection of *Salmonella* and verocytotoxigenic *E. coli* in food.

The latest work on *Salmonella* and verocytotoxin producing *E. coli* detection using gene probe methods has been surveyed. Commercial kits for PCR detection of *Salmonella* spp. have been tested. Where suitable kits were unavailable, primers were synthesised and optimised for PCR. The limits of detection for all primer pairs have been determined using (a) purified DNA and (b) dilutions of cell suspensions of pure cultures. Limits of PCR detection in enrichment broths have been determined by adding dilutions of target organisms to enrichment broths pre-incubated with a range of food types. The effectiveness for PCR of different methods of extracting DNA from enrichment broths have been compared.

FS1266 (B09009): Develop and validate standard method to detect *Cryptosporidium cyclospora* and other parasitic protozoa on fruit and vegetables.

A method had been developed to remove *Cryptosporidium* oocysts from lettuce prior to IMS concentration and IF microscopy.

FS1267 (B090010): Develop and validate an immunological method for the detection and characterisation of all VTEC in foodstuffs.

An enrichment procedure for detection of VT1, VT2, O157 somatic antigen and other selective virulence factors has been optimised.

FS1268 (B090011): Develop a rapid and specific PCR method to detect *Clostridium botulinum* spores in food and environmental samples.

A method for spore extraction from food matrices and a PCR detection method for use with foods have been developed. The selectivity and sensitivity of the PCR detection method have been compared with alternative detection methods.

3. Dissemination Activity

Results from projects commissioned in the Programme are regularly reported in peer-reviewed academic journals, at conferences and at national and international meetings. In addition, there have been briefing meetings with various groups with policy responsibilities. A Programme workshop was held in October 1999 at the Centre for Applied Microbiology and Research, Porton Down, Salisbury at which project leaders presented their aims and results to an audience comprising representatives from MAFF, Universities and Food Research Associations and Institutes.

4. Future Activity

No more research will be commissioned under Programme FS12 and all current research projects will be transferred to the Food Standards Agency.

5. Comments

The Programme Adviser in 1999 was Dr Chris Bell.

FS12 (B09) – Detection and separation of food pathogens

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS1223 (B09001)	A method for detecting <i>Cryptosporidium parvum</i> oocysts and <i>Giardia duodenalis</i> cysts in natural mineral waters	Scottish Parasite Diagnostic Laboratory	01/05/95	30/09/99	29,827
FS1242 (B09002)	Rapid detection, quantification and molecular characterisation of thermophilic campylobacters in foodstuffs	Public Health Laboratory Services	01/09/96	31/08/99	23,213
FS1245 (N/A)	Sono-chemical signal development as a novel generic method for rapid, <i>in situ</i> and specific detection	University of Manchester	01/07/96	30/06/99	59,710
FS1249 (N/A)	Development of assays for <i>Clostridium botulinum</i> toxins in food	Centre for Applied Microbiology and Research	01/04/96	30/09/99	113,699
FS1250 (B09003)	Virus survival in food matrices	University of Surrey	01/04/97	31/03/00	66,861
FS1260 (B09006)	Preparation of 'validation samples' for use in the evaluation of microbiological methods	Central Science Laboratory, York	01/04/98	31/01/00	83,900
FS1264 (B09007)	Develop & validate methods to detect & characterise verocytotoxin producing <i>Escherichia coli</i> in foods	Public Health Laboratory Services	01/09/99	31/08/02	32,341
FS1265 (B09008)	Accelerated detection of salmonella and verocytotoxigenic <i>E.coli</i> in food	Reading University	01/06/99	31/05/01	36,127

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS1266 (B09009)	Develop & validate standard method to detect <i>Cryptosporidium cyclospora</i> & other parasitic protozoa on fruit & vegetables	Scottish Parasite Diagnostic Laboratory	01/04/99	30/09/01	119,495
FS1267 (B09010)	Develop & validate an immunological method for the detection & characterisation of all VTEC in foodstuffs	IFR, Institute of Food Research	01/02/99	31/05/02	74,712
FS1268 (B09011)	Develop a rapid & specific PCR method to detect <i>Clostridium botulinum</i> spores in food and environmental samples	Central Science Laboratory, York	01/04/99	31/03/00	100,000
TOTAL					739,885

FS15 (now B07) – Growth conditions for pathogens

1. Background

The elimination of all pathogens from the food supply, although an ideal solution, is currently unrealistic in the face of consumer demand for an increased supply of lightly processed foods. In practice, the microbiological quality of the food chain should be improved by the rigorous application of properly developed hazard analysis and critical control point (HACCP) procedures.

The aim of this programme was to understand how environmental conditions and intracellular mechanisms interact to control the growth, survival and, where applicable, toxin production of current and emerging bacterial pathogens in food. It included studies on both undamaged bacteria and those injured by food treatment processes. The application of this knowledge in HACCP should increase its effectiveness and properly applied be expected to lead to an improvement in food safety.

The programme began in 1993 and finished in 1999 when the last projects were completed. The cost for this programme in Financial Year 1999/2000 was ca. £154k.

2. Outputs

The main outputs from this Programme have been described in previous Annual Reports. Only five projects remained to be completed in 1999 and the following report concerns these.

Attempts have been made to define food environments that would uncouple toxin production from growth so that even if growth of a pathogen occurred accidentally in a food, toxin production would be minimised. This was predicated on a finding that the expression of virulence genes in *Listeria monocytogenes* could be repressed by the addition to the growth medium of cellobiose or arbutin. However, an investigation of the effects of environmental and nutritional factors on growth and toxigenesis in *Bacillus cereus* under controlled conditions in batch and continuous culture has not uncovered conditions that would seriously diminish production of either the emetic or diarrhoeagenic toxins (FS1530). In a study of environmental conditions that affect neurotoxin production in a Group II, non-proteolytic, toxigenic strain of *Clostridium botulinum* the genetic transformation of such a strain, ATCC25765, was demonstrated for the first time (FS1529). This allowed the introduction of reporter genes coding for β -galactosidase or Lux proteins, under the control of the neurotoxin promoter, into the *Cl. Botulinum* strain which could then be used to assay toxin formation indirectly.

Methods have been developed for studying the effects of novel food treatment processes on bacterial membrane activity (FS1532). The application of pulsed electric fields causes the bacterial membrane to leak UV-absorbing material but cell inactivation shows an all or none effect i.e. cells survive in an uninjured mode or are killed; there is no injured population. It is apparent that both the membrane composition (fluidity) and bacterial cell size (diameter) are important in determining the sensitivity of populations to pulsed electric fields. The application of high pressure to bacteria on the other hand, induced both cell death and sublethal injury, the latter population being able to recover and grow at the same rate as non-treated cells. The membrane does not appear to be a major site for inactivation by high pressure.

The application of high hydrostatic pressure to food materials as a “pasteurising” treatment is already commercial. Mutations in *Escherichia coli* have been identified that result in increased or decreased resistance to high pressure treatment. In addition treatments that cause enhanced sensitivity or resistance have been identified. There was wide variation in pressure resistance between natural isolates of both *E. coli* O157 and *Listeria monocytogenes* (FS1531). Increased pressure resistance was associated with the stationary phase of growth, a slow growth rate, sub-lethal pressure-shock, heat shock or exposure to spent medium. The strain differences in pressure resistance in natural isolates of *E. coli* O157 were due to differences in expression of the *rpoS* stationary phase global

regulatory system. This project should enable sensible guidelines to be produced governing the use of high pressure to reduce the viability of bacteria such as *E. coli* O157 in treated products.

Under suitable conditions sub-lethally injured cells can repair cell damage and recover all their normal properties including virulence. Conversely, holding injured cells under conditions that prevent repair can result in a further loss of viability that may be exploited in designing combination treatments to preserve food. The effects of different stress treatments and post-injury holding conditions on viability of *E. coli* and *L. monocytogenes* have been investigated using conventional plate-counting methods combined with fluorogenic or chromogenic assays of membrane integrity and metabolic activity (FS1534). It became clear that cells that show very little metabolic activity are not necessarily dead and, conversely, that other treatments allow cells to retain membrane integrity and metabolic activity in cells that are unequivocally dead. The only way to demonstrate that a cell is alive is to show that it can multiply and this entails finding the optimum recovery conditions empirically.

3. Dissemination Activity

Results from the programme have been widely disseminated in the scientific literature and by presentation at colloquia, workshops and national and international meetings. A programme workshop was held in November 1999 at the Campden & Chorleywood Food Research Association, Chipping Campden at which project leaders presented their aims and results to an audience comprising representatives from MAFF, the Food Industry, Department of Health, Universities, Food Research Associations and Institutes. Speakers were encouraged to emphasise the contribution of each project to MAFF policy requirements while representatives of the food industry with relevant experience were invited to become more involved with the contracting laboratories.

4. Future Activity

The last projects in this Programme were commissioned in 1996 and ended early in the 1999/2000 financial year. FS15 was replaced by programme FS31 "Assessing Microbiological Hazards and Risks".

5. Comments

The Programme Adviser was Professor Bevan Moseley OBE.

FS15 (now B07) – Growth conditions for pathogens

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS1527 (N/A)	Role of natural food constituents (cholines and peptone) in the growth, survival and resuscitation by pathogenic bacteria	University of Aberdeen	01/08/95	05/02/99	4,150
FS1529 (B07001)	Development of gene reporters to aid in the analysis of the physiological factors which affect botulinum toxin product	Centre for Applied Microbiology and Research	01/05/96	31/07/99	17,295
FS1530 (N/A)	Factors controlling toxin formation by <i>Bacillus cereus</i> and other psychrotrophic <i>Bacillus</i> spp	Hannah Research Institute	01/10/96	30/11/99	44,511
FS1531 (N/A)	Mechanisms of microbial resistance to high hydrostatic pressure	IFR, Institute of Food Research	01/04/96	30/06/99	11,329
FS1532 (N/A)	Characterisations of bacterial membrane damage associated with electric field and high pressure treatment	Campden & Chorleywood Food Research Association	01/09/96	31/08/99	64,488
FS1534 (N/A)	Metabolic capacity and viability of injured cells surviving novel foods preservation methods	IFR, Institute of Food Research	01/04/96	30/06/99	12,295
TOTAL					154,068

FS31 (now B01) – Assessing microbiological hazards and risks

1. Background

The objectives of this programme are to increase our understanding of how and where pathogenic microorganisms enter the food chain and to assess how specific food handling and production processes affect the survival, growth and toxin formation of microorganisms in food. The aim is to enable Government and industry to assess objectively the hazards and risks from foodborne pathogens and to assess the significance in consumer safety

terms of changes in food production processes and the consumption habits of the UK population.

This programme began in 1997 and will finish in 2002. The cost for this programme in Financial Year 1999/2000 was ca. £808k.

2. Outputs

The effect of microstructure and the microscopic distribution of water on bacterial growth and survival are being investigated using microstructured growth media consisting of randomly packed arrays of Sephadex microspheres and phase separating dextran of low and high molecular weight (FS3101). The microstructural effects of the Sephadex were considerable e.g. no survivors of *Salmonella typhimurium* after 50 hours compared with a 2 to 3 log reduction in survival in broth medium at the same water activity after 300 hours. Current mechanistic microbial growth models have been reformulated leading to a prediction of the distribution of lag times in a bacterial population and as a consequence the size of colonies resulting from growth. This is important from a practical point of view because the cells in a population having the shortest lag will have the greatest impact on food safety. Measurements of the lag times of individual cells of *Escherichia coli* under stressed and non stressed conditions are currently being accumulated. With *E. coli* there is much less scatter of lag times from cultures started from very small inocula than is experienced with *Listeria monocytogenes*.

A number of projects are concerned with the effect of food-processing stresses on bacterial populations and the possible emergence of strains that are more resistant to the stresses or more virulent when consumed. An excellent method has been developed for the typing of isolates of *Salmonella enteritidis* using fluorescent AFLP (amplified fragment length polymorphisms) (FS3111). A large number of isolates has now been typed and will be subjected to heat, acid and drying regimes on a cyclical basis to identify changes in the population. After six cycles of heating and regrowth of heat-sensitive and heat-resistant strains, resistance increased in both sensitive and resistant phenotypes. A mutator strain has been isolated and will be used to investigate possible connections between mutator strains and the emergence of resistant or virulent strains during processing. Exposure of salmonellae to chill temperatures (6°C) for four weeks significantly increased their tolerance to acid while exposure to repeated cycles of acid stress (pH2.8 for 30 min) increased resistance to pH2.8 but not 2.5. Prolonged exposure to cold stress decreased tolerance to low pH and heat (FS3112). The technique of fluorescent AFLP is being applied to two other foodborne pathogens *Salmonella typhimurium* DT104 (FS3114) and *E. coli* O157 (FS3115) with a view to identifying the sources of human infection. These projects are in their early stages and have yet to achieve the level of

discrimination necessary to determine relationships between environmental, animal and human isolates. The ability to be able to differentiate between tolerant and sensitive isolates of *Salmonella* on the basis of simple, standardised tests is proving successful. There appears to be a good correlation between the formation of convoluted colonies on brilliant green agar at 25°C and tolerant forms. The apparent differences between heat and acid resistance of strains grown in various complex media is due to the presence/absence of glucose in the media. The presence of glucose causes a reduction in pH which increases both heat and acid tolerance (FS3109).

Organisms such as *S. typhimurium* respond to harmful environments by activating stress responses which result in an altered profile of protein synthesis so that it should be possible retrospectively to determine that such stresses have been experienced. The post-stress detection of cold and hypochlorite induced stress proteins is being investigated (FS3110). Using 2-D gel electrophoresis increased expression of selected proteins has been observed post-stress for both exponential and stationary phase cells and their identity and use as markers are being investigated.

In recent years there has been a dramatic increase in the amount of available data on microbial responses to various stresses encountered in the food chain. A systematically formatted database on such responses of foodborne pathogens, called MicroBase, is being established (FS3113). Such stresses include physical ones such as heating, chilling, freezing, drying and chemical stresses such as food preservatives and naturally-occurring antimicrobial agents. It is expected that about 20,000 records will be incorporated in the database.

An assay has been developed using human foetal intestinal INT407 cells to measure the invasiveness of *Campylobacter jejuni* isolates from poultry, poultry housing and surrounding environments (FS3107). Over 100 isolates have been tested to date. The most highly invasive strain is 200 times more invasive than other strains. No obvious correlation has been found between the source of isolation and invasiveness. Preliminary results show that invasiveness decreases when the bacteria are exposed to the environmental conditions experienced during poultry processing.

Strategies to reduce food poisoning need to be developed and evaluated based upon risk assessment. Recently highly-structured quantitative risk assessments have been used to assess the risks associated with specific hazards, e.g. *S. enteritidis* and eggs in the USA, throughout the food chain. The overall aim of one project (FS3116) is to develop a method for determining exposure assessment of risk associated with *Salmonella* and *Campylobacter* following the preparation and consumption of poultry products in the home and food service establishments. Levels of

contamination of the poultry and its packaging have been measured and preparation behaviour monitored by video and notational techniques.

The influence of various characteristics of polymer films on the ability of microbes to adhere to them has concentrated on the lower critical solution temperature (LCST) of the polymeric material. Bacterial adhesion has been shown to vary with the T_{LCST} of N-isopropyl acrylamide polymers on glass slides but the variation is less than might have been anticipated e.g. generally three fold differences but with occasional results in excess of eight-fold (FS3108).

6. Dissemination Activity

Results from this programme are being disseminated in the scientific literature and by presentation at colloquia, workshops and national and international meetings. A programme workshop was held in November 1999 at the Campden & Chorleywood Food Research Association, Chipping Campden at which project leaders presented their aims and results to an audience comprising representatives from MAFF, the Food Industry, Department of Health, Universities, Food Research Associations and Institutes. Speakers were encouraged to emphasise the contribution of each project to MAFF policy requirements, while representatives of the food industry with relevant experience were invited to become more involved with the contracting laboratories.

7. Future Activity

No more research will be commissioned under programme FS31 and all current research projects will be transferred to the Food Standards Agency.

8. Comments

The Programme Adviser is Professor Bevan Moseley OBE.

FS31 (now B01) – Assessing microbiological hazards and risks

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS3101 (B01001)	Physiological and microstructural factors controlling the survival and lag of food borne pathogens	IFR, Institute of Food Research	01/04/97	31/08/00	90,418
FS3102 (B01002)	Kinetics of inactivation of micro-organisms by high voltage electric pulses, alone and in combination	Reading University	01/08/97	31/07/00	58,921
FS3103 (N/A)	The evaluation and control of Biofilm of Significance to the food industry	University of Wales, Cardiff	01/09/97	28/02/99	9,775
FS3104 (B01003)	Characterisation of the non-linear thermal inactivation kinetics observed with <i>Mycobacterium paratuberculosis</i>	Queen's University of Belfast	01/11/96	31/10/00	38,793
FS3107 (B01005)	Variations in the virulence of <i>Campylobacter jejuni</i> strains associated with poultry and poultry meat	Veterinary Laboratories Agency	01/04/98	31/03/01	66,043
FS3108 (B01006)	Why do bugs stick to what they stick to?	IFR, Institute of Food Research	01/04/98	31/03/01	33,331
FS3109 (B01007)	Development & study of tests to differentiate between tolerant & sensitive isolates of <i>salmonella</i> & <i>E. coli</i> O157	Public Health Laboratory Services	01/07/98	30/06/01	50,260
FS3110 (B01008)	Post stress detection of cold and hypochlorite stress proteins in <i>Salmonella typhimurium</i>	University of Edinburgh	01/10/98	03/11/01	48,460
FS3111 (B01009)	Assessment of population changes in <i>Salmonella enteritidis</i> & the emergence of strains with altered properties during food production	IFR, Institute of Food Research	01/04/98	31/03/01	99,911

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS3112 (B01010)	Evaluation of risk of induction & selection of more stress tolerant & virulent <i>salmonellas</i> by exposure to food production-related stress	Centre for Applied Microbiology and Research	01/07/98	31/03/01	17,446
FS3112 (B01011)	Evaluation of risk of induction & selection of more stress tolerant & virulent <i>salmonellas</i> by exposure to food production-related stress	Public Health Laboratory Services	01/07/98	31/03/01	53,597
FS3113 (B01012)	Dynamic database on microbial responses to common stresses in the food chain	IFR, Institute of Food Research	01/04/99	31/03/02	64,671
FS3114 (B01013)	Genotypic subtyping of multiresistant <i>Salmonella typhimurium</i> DT 104 from food animals and humans	Public Health Laboratory Services	15/06/99	14/06/01	39,875
FS3115 (B01014)	Development of a novel molecular typing method for comparison of food-borne pathogens using VTEC as a model organism	Laboratory of the Government Chemist	01/05/99	30/04/01	103,941
FS3116 (B01015)	Determine exposure assessment & modelling risks associated with the preparation of poultry – catering & home	University of Wales Institute, Cardiff	01/06/99	30/06/01	32,245
TOTAL					807,687

FS32 (now B02) – Managing microbiological hazards and risks

1. Background

Food contamination is an on-going subject which causes great public concern. The majority of problems involve microbiological contamination incidents, which in many cases are preventable.

The B02 programme was devised to investigate and develop practical strategies that can be used by government, the food industry and consumers, to prevent, control or eliminate such contamination.

The programme commenced in 1997 and will be completed by mid 2001. The expenditure in the year 1999–2000 was ca. £670k and the cost of the programme overall is ca. £3 million. The 10 projects deal with various food safety management issues involving scientific, technological and human behavioural factors. The use, mis-use of and problems associated with HACCP are studied particularly.

2. Outputs

One project considered whether the current thermal treatments used in the food industry to bring about defined reductions in numbers of *E.coli* O157 are adequate. In particular, whether the ACMSF report on VTEC recommending that minced beef and related products should be heated to an internal temperature of 70°C for 2 minutes or equivalent was adequate in the light of other research on sub-lethally damaged cells. A conclusion from this study is that for practical purposes the ACMSF recommendations are valid.

The development of a computer software package in the design of a comprehensive safe food process was the feature of a further project. This package is now being marketed at £550 + vat providing food businesses with an on-site, easily accessible food safety assurance tool.

Innovative cleaning and de-contamination technologies for cleaning fruit and vegetables using ultrasound and photodynamic methods and the investigation of the practicality of a “cold jet” technique, employing pellets of CO₂ dry ice (a practice used to strip paint from aircraft) to clean food process surfaces, have been employed in 2 projects. Results indicate that cleaning and decontamination of foods and food preparation surfaces can be achieved by these methods, either singly or in combination with more traditional approaches.

Knowledge, beliefs, attitudes and actual behaviour of food handling individuals in domestic and commercial food production circumstances have

been studied in 2 projects. What has been revealed to date is that although knowledge of correct practices may be present, under actual examination this knowledge is not always used to prevent contamination of either food or preparation surfaces. Aspects of this research need to be taken forward into the development of relevant educational and training tactics and methods.

In 3 projects, the use of HACCP, the necessity for documentation and the degree of verification of HACCP procedures required, have been studied. In the first of these which concentrates on meat product manufacturing and butchers' shops, barriers to the adoption of HACCP were identified as being the lack of appropriate training and access to expert advice.

Some companies had relied on independent consultants, without obtaining good results, but where experienced technically qualified in-house personnel were employed, standards and achievement of HACCP were much improved.

The second project involved companies manufacturing a range of dairy, drink, sandwiches and other products. Most of them could identify hazards but had difficulty identifying critical control points (CCPs). This was not aided by a general lack of consistency in judging HACCP by customers, auditors and EHOs. In general, there was found to be much confusion and lack of understanding regarding the verification of HACCP.

The third project, concentrating on catering and retail sectors, has found an excess of documentation in applying HACCP but again a general failure to identify and focus on CCPs correctly.

Possible improvements so far identified in these studies include:-

- improved dissemination of technical information for food businesses,
- emphasis on the differences between quality and safety issues,
- reducing the number of CCPs by expert risk assessment,
- effective identification of general hygiene aspects (GHP) from the precise product/process controls required for HACCP.

An objective is to establish a documentation system which acts to direct and filter the problem solving process in order to arrive at valid CCPs.

3. Dissemination Activity

Results from the research studies within the programme have been the subject of individual project workshops and seminars and published articles in national and internationally recognised scientific and technical journals.

A programme workshop was held in March 2000 at the Royal Institute of Public Health & Hygiene & Society of Public Health in London, when 100 delegates from food companies, enforcement bodies and food hygiene training and educational establishments in addition to government department representatives, attended.

An international food safety publication featured an introductory article on the FS32 – now B02 – programme, and a detailed article on one of the projects. Further projects will be reported on by the publication in the year 2000–2001.

4. Future Activity

All the projects will be completed by the 30 June 2001 and there are no plans to continue with the Programme in its current form – based on such a wide ranging and general approach to the management of microbiological hazards and risks. A further workshop is planned for the north of England later in 2000 which will concentrate on HACCP and food safety educational aspects.

The Food Standards Agency will be examining closely the results of the valuable research carried out within the programme as much of it is directly related to the functions of the Agency itself. The effective control and management of the microbiological causes of food borne illness is a necessity for government and the Agency, the food industry and consumers in their own homes.

Information produced by B02 therefore, will with some certainty contribute significantly to the Food Standards Agency's work in the areas of risk communication and food safety enforcement.

5. Comments

The Programme Advisor in 1999–2000, Mike Jacob, will produce an end of programme report outlining the conclusions from research carried out and recommendations for further areas of work related to the management of safety in food handling and production.

FS32 (now B02) – Managing microbiological hazards and risks

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS3202 (B02001)	Thermal death of pathogenic micro-organisms in real foods	IFR, Institute of Food Research	01/05/97	30/04/00	77,852
FS3203 (B02002)	Barriers to the adoption of good hygiene practice by small and medium-sized food manufacturers	Campden & Chorleywood Food Research Association	01/10/97	31/03/00	27,373
FS3203 (B02003)	Barriers to the adoption of good hygiene practice by small and medium-sized food manufacturers	Reading University	01/10/97	31/03/00	53,832
FS3204 (B02004)	An evaluation of food handlers knowledge beliefs & attitudes about food safety & its interpretation using social cognitive models	University of Wales Institute, Cardiff	01/07/98	30/06/01	40,017
FS3206 (N/A)	Efficacy testing of disinfectants used in the food industry against a range of pathogens including <i>E. coli O157</i>	Laboratory of the Government Chemist	01/05/98	31/10/99	63,968
FS3208 (B02005)	Novel techniques for cleaning and decontaminating raw vegetables and fruit	Campden & Chorleywood Food Research Association	01/04/98	31/03/00	127,366
FS3209 (B02006)	Cold Jet – A novel technique for cleaning & decontaminating food processing areas, equipment, carcasses and foods	Food Science & Technology Research Centre	01/02/99	31/12/01	64,991
FS3210 (B02007)	Assessing and reducing the risk of cross contamination of food stuffs in food handling environments	University of Wales Institute, Cardiff	01/01/99	31/12/00	55,469

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS3212 (B02009)	Assess the documentation & degree of verification required to minimise the burden of HACCP on SMEs yet ensure safety	ADAS Consulting Ltd	01/05/99	31/10/00	60,658
FS3213 (B02010)	The evaluation & application of information on consumer hazard & risk to food safety education	University of Wales Institute, Cardiff	01/07/99	30/06/01	53,250
FS3214 (B02011)	Develop generic models for documentation and verification as a means of reducing the burdens of HACCP on SMEs	University of Central Lancashire	01/05/99	31/10/00	45,807
TOTAL					670,583

FS33 (now B03) – Assessing and managing the hazards and risks from *Campylobacter* spp. and *Salmonella* spp. in poultry from farm to fork

1. Background

Hygienic practices on poultry farms and throughout processing, distribution and retailing have been intensified in recent years. As a consequence, contamination of raw poultry with salmonellae has been reduced but contamination with campylobacters is common. This programme is intended to identify reliable and cost-effective measures that can be used at all points in the food chain, from farm to fork, to reduce carriage of *Campylobacter* spp. and *Salmonella* spp. from poultry produced in the UK or to reduce their prevalence to the lowest level possible. The programme includes research projects, reviews of current knowledge and visits to poultry processors to determine:

- how and where *Campylobacter* spp. and *Salmonella* spp. enter the food chain,
- how specific food handling and production processes that affect the survival and growth of *Campylobacter* spp. and *Salmonella* spp. can be assessed,

- where further information about the hazards and risks can be identified and prioritised,
- whether novel approaches might reduce contamination in poultry products,
- how practical techniques appropriate for use by government, industry and consumers to manage the hazards and risks arising from the presence of *Campylobacter* spp. and *Salmonella* spp. in poultry and poultry products can be developed.

The total spend on B03 in FY99-00 was *ca.* £272k.

This programme compliments the element in the LINK programme on Advanced and Hygienic Food Manufacturing concerned with hygienic assurance.

2. Outputs

Eight projects were funded in 1999–2000.

Field studies to identify and evaluate key intervention points for *Salmonella* control during broiler production (FS3301, ends in 2000) is visiting selected UK broiler producers and sampling chicks, birds, feed and their environment through production, transportation and the abattoir. The origins and types of salmonellae detected in the environment are compared with those on the carcasses. Key intervention points for control of salmonellae are being identified and tested under commercial conditions.

Poultry meat is widely believed to be an important source of campylobacters causing gastroenteritis in man, although conclusive evidence is lacking. The molecular epidemiology of campylobacters in poultry and poultry meat (FS3303, ends in 2000) is attempting to identify the origins of campylobacters colonising poultry and to develop intervention strategies. Campylobacters isolated from a range of sources including man, the environment and poultry, are being used to develop a sub-typing scheme to assist tracing the origins of campylobacters found on poultry. Chicks are commonly free from campylobacters, but if a few birds become colonised, the whole flock becomes colonised within a few days. If the origin of that contamination could be identified, it might be preventable.

The project to identify critical points for infection of live birds or contamination of poultry carcasses with campylobacters and salmonellae (FS3306, ends in 2002) is complementing previous efforts in FS3301 and FS3303 by paying particular attention to the role of aerial contamination. In addition, a wide range of sites, many not included in earlier studies, are

being sampled e.g. crates used to transport live birds on arrival at the processing plant. Biosecurity is being documented in detail in an attempt to identify practices that help to limit carcass contamination.

In another complementary project (FS3308, ends in 2001) the efficacy of water disinfection systems for broiler production units is being investigated. When tested by the most sensitive bacteriological methods, drinking water in the shed is often contaminated with campylobacters. It is uncertain whether this contamination occurs in holding or header tanks, or is merely contamination of the nipple drinkers by the birds. Again, management systems are being documented in detail in an attempt to identify best practices.

3. Dissemination Activity

A fifth joint workshop with LINK on Hygienic Food Processing for Engineers and Microbiologists was held on 11 January 2000 in London. Lectures included Localised cooling (AFM3), Detection of volatiles via fly antennae (Bridge LINK), Slippery surfaces to prevent biofilms (FS1047), Food structure and microbial growth (FPS123), Microbial inactivation during food processing (BBSRC), Risk assessment – a network approach (FS1046), Effective decontamination processes (FS1043, MAFF Fellowship, EU), Novel high oxygen and noble gas modified atmosphere packaging (FAIR Shared Cost CT96-1104). In addition posters represented projects in LINK, FS10, FS15, FS32 and Shared Cost CT97–3129.

Results from the programme are being disseminated in the scientific literature and by presentation at colloquia, workshops, national and international meetings.

4. Future Activity

With transfer of responsibility to the Food Standards Agency, the future direction of the programme will be modified to take account of results obtained and new opportunities. A workshop for those active in research in this area and representatives of the poultry industry is being planned for late 2000.

5. Comments

Feedback from delegates who attended the Fifth Workshop on Hygienic Food Processing shows continued and wide interest in all aspects of research to ensure food safety. The workshops have resulted in several new collaborations between different scientific disciplines.

The Programme Adviser is (Dr) Terry Roberts OBE, Food Safety Consultant.

FS33 (now B03) – Assessing and managing the hazards and risks from *Campylobacter* spp. and *Salmonella* spp. in poultry from farm to fork

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS3301 (B03001)	Field studies to identify and evaluate key intervention points for salmonella control during Broiler production	Veterinary Laboratories Agency	01/07/97	30/06/00	67,396
FS3302 (B03002)	Risk factors of cross infection by <i>Salmonella</i> spp. from fresh poultry packaging in retail stores	Campden & Chorleywood Food Research Association	01/09/98	30/11/99	30,045
FS3303 (B03003)	The molecular epidemiology of campylobacters in poultry and poultry meat and use to develop intervention strategies	Veterinary Laboratories Agency	01/04/97	31/03/00	87,004
FS3305 (B03006)	Review of measures to reduce levels of Salmonella and Campylobacters in poultry & development of an appropriate risk assessment model	ADAS Consulting Ltd	01/09/98	31/08/99	19,632
FS3305 (B03005)	Review of measures to reduce levels of Salmonella and Campylobacters in poultry & development of an appropriate risk assessment model	Silsoe College	01/09/98	31/08/99	33,773
FS3305 (B03007)	Review of measures to reduce levels of Salmonella and Campylobacters in poultry & development of an appropriate risk assessment model	Nottingham University	01/09/98	31/08/99	8,835
FS3306 (B03008)	Identify critical points for infection of live birds or contamination of poultry carcasses with campylobacter & salmonella	Public Health Laboratory Services	01/11/99	30/10/02	17,230

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
FS3308 (B03010)	Efficiency of water disinfection systems broiler production units	University of Aberdeen	01/10/99	31/03/01	7,980
TOTAL					271,895

MH02 (now M01) & M03 – Meat hygiene research

Overview

The public rightly demands that hygiene standards in fresh meat slaughterhouses and cutting plants are of the highest order. However for a variety of reasons (technical, economic etc) it may never be possible to completely eliminate all human pathogens from fresh meat and fresh meat products. Despite this, information resulting from the research programmes can set the context in which decisions are made and resources allocated.

The Meat Hygiene research programmes are directed towards the protection of the public by promoting food safety, and are comprised of two strands covering microbiological safety (M01) and transmissible spongiform encephalopathies (TSEs) (M03). To achieve this objective the focus of the research on the microbiological safety of meat is on a better understanding of hygiene hazards of which pathogenic enteric bacteria are the most important. The output from the research programme is used to identify hazards and control points in the meat production chain that can be used in the development of HACCP systems. The TSE (Food Safety) programme targets risk, public health and susceptibility to infection (other food producing species).

These risk-based controls will be included, in the future, in EU food hygiene legislation following the ongoing review of food hygiene directives by the EU Commission. This follows worldwide moves (CODEX Alimentarius) to introduce risk-based legislation better suited to the process control of pathogenic microorganisms.

The cost of the meat hygiene research programme in the 1999/00 Financial Year was *ca.* £465K, whilst the TSE (Food Safety) programme was *ca.* £2.6 million.

MH02 (now M01) – Meat hygiene (microbiological safety)

This programme is divided into three strands; **Contamination**, **Engineering Solutions** and **Risk Assessment**. The distribution is a reflection of the priority the Agency attaches to each of these areas, and as a result the emphasis of the programme has moved away from engineering solutions and more towards control of contamination and risk assessment.

Contamination

The objective within this strand is to obtain a greater understanding of the distribution of pathogenic bacteria within the abattoir environment and meat plants and to devise techniques for reducing pathogenic microorganisms.

The main thrust of the research is directed towards the abattoir environment where it is considered that the greatest risk of contamination lies, and therefore where the greatest impact on meat hygiene can be achieved. Studies have focused on the identification of the major sources of microbiological contamination with a view to developing practical solutions to the reduction of contamination. However it is acknowledged that other areas of the meat production/supply chain can also influence the microbiological status of meat.

- MH0226. This study is comparing changes in procedures, slaughterhouse environment and facilities, and their impact on the microbial status (pathogenic and non-pathogenic microorganisms) of carcasses. The study involves close liaison with the industry and is paying particular attention to slaughter procedures and microbiological sampling protocols for carcasses and the slaughterhouse environment. The results obtained so far indicate that the main source of contamination occurs during evisceration from the hands of the slaughterman, due to failure to wash hands thoroughly following tying off of the rectum. Aerosols generated during the slaughter process have also been identified as a significant source of contamination and cross contamination. The results also show that the effect of feed withdrawal prior to slaughter lead to changes in the pH of the gut contents of animals that might in turn lead to a selective increase of certain microorganisms.
- MH0233. The objective for this study is to provide guidance on best husbandry practice for the production of cattle with low visible contamination and microbiological contamination. The study will focus on a variety of feed regimes and will include diet changes and withdrawal immediately prior to

transport to market or slaughterhouse. As the study commenced in early 2000, it is too early for results to be reported.

- MH0229. This study is examining the spread of microbiological and physical contamination between cattle during the farm to abattoir phase of the production cycle. Success of the project depends heavily on the development of a reliable and reproducible sampling protocol and early studies have focused with some success on this issue. The presence of a range of microbial contaminants has been monitored and initial results indicate that *Enterobacteriaceae* and *E.coli* levels were higher pre-transport on “dirty” cattle than “clean” cattle, with the highest level of contamination present on the brisket. The research has also shown that weather conditions can have a significant influence on the level of contamination. However more data together with a detailed analysis of the results will be required before any firm conclusions can be drawn.
- MH0217. Studies on the relationship between feed withdrawal and processing hygiene in poultry have found that withdrawal of feed results in a reduction in the frequency of defaecation and water consumption. However moisture content of the gut contents increases with longer withdrawal periods, as do the numbers of *Enterobacteriaceae* and *Campylobacters*. The researchers also noted that the strength of the gut was reduced, thus increasing the risk of contamination by gut contents during processing.
- MH0230. In support of the “Clean Livestock Policy” a short feasibility study was commissioned to examine the potential for wood chips to offer an alternative to straw as a bedding substrate for beef cattle. The results although not conclusive suggest that this substrate over a period of 4 weeks has the potential to match the cleanliness of cattle housed on straw. Beyond 4 weeks the cleanliness scores increase. The results offer options for future studies in this area.
- MH0231/MH0235. These two studies have recently commenced and are complementary. They aim to identify factors effecting the MHS cleanliness score for sheep arriving at abattoirs, the level of carcass bacterial contamination following processing and to develop intervention procedures to improve the MHS scores and reduce carcass contamination. The studies will cover all areas of the production cycle (farm, transport, and market, seasonal and geographic) and include a survey of

carcass bacterial contaminants (*E.coli* O157:H7, *Salmonella*, *Campylobacter*, total coliforms and *E. coli*). Preliminary results of the microbiological sampling programme have shown that *E.coli* O157:H7 and *Campylobacter*, but not *Salmonella* are present in the bedding of livestock transporters. However the data set is limited and the true picture will not be evident until the sampling and analysis programme is completed. In the slaughterhouse the picture is clearer, the data clearly indicating that of the two sites sampled on the carcass (shoulder and brisket), bacterial contamination is highest on the brisket.

- MH0232. An earlier research project that investigated the effect of chlorinated water on poultry carcasses, demonstrated that halomethanes were not present at concentrations greater than that permissible in potable water (there are no limits set for meat). However that study did not provide any information regarding the possible formation of chlorinated cyclicimides (maleimides), which are known to be mutagenic. The purpose of the current study (MH0232) is to investigate the possible formation of these compounds, to develop and validate analytical methods and to determine their concentration in poultry tissues. This project has not reached a stage where results can be reported.

Engineering Solutions

No research was commissioned in this area during the reporting year.

Risk Assessment

The applied science of risk analysis is gaining in importance to meat hygiene as it may identify where resources and effort need to be applied to improve the status of meat and meat products both in domestic and international trade. Risk analysis techniques are being increasingly applied to assessing the validity of post mortem meat inspection programmes.

The objective of this strand of the meat hygiene research programme is to assemble information, which will enable quantification of the biological hazards associated with meat. The intention in the longer term is that this will permit prediction of changes in the incidence of adverse effects on the human population, from a knowledge of any proposed changes in meat hygiene inspection procedures.

In addressing these general objectives the meat hygiene research programme has been directed towards obtaining a better understanding of the nature and extent of the distribution of pathogenic microorganisms from

the farm to the abattoir. In particular, the programme aims to provide data to underpin the development and application of HACCP systems in the production of fresh meat from “farm to fork”.

- MH0224. A review of microbiological contamination of poultry carcasses, control measures and the potential for risk assessment modelling had as one of its objectives the consolidation of information published in peer review journals. However it was evident that there is a paucity of published information and that which was available was variable in quality and was of little real value. In particular most of the reported data only provided information on the incidence (positive or negative) and did not include levels of contamination. In addition it was noted that the diversity of sampling methods also increased the variability of the data. It was concluded that there was insufficient information to permit the development of a risk assessment model.
- MH0220. Studies aimed at establishing the Critical Control Points for enteric pathogens in pork production began during 1999. Monitoring the microbiological contamination on pig carcasses at specific points in the slaughter process the results show that as expected there is a significant reduction (~100 fold) in contamination post-scalding, but that carcasses become re-contaminated during scraping and evisceration. The main contamination and re-contamination risk factors identified were the worktable surfaces, scraper blades and the hands of the butcher and knives used to eviscerate the carcass.

Future Research

The research program will continue to focus on the strands described above. Research is being commissioned on risk assessment and alternative husbandry methods to reduce microbiological contamination of livestock entering slaughterhouses.

MH02 (now M01) – Meat hygiene (microbiological safety)

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
MH0217 (M01001)	The relation between feed withdrawal in broilers and meat hygiene	Bristol University	01/04/96	31/03/99	13,253
MH0220 (M01002)	Establishment of critical control points for enteric pathogens in pork production	Nottingham University	15/03/99	14/03/01	82,349
MH0224 (M01004)	A review: Microbial contamination of poultry carcasses, control measures and scope for risk assessment modelling	Silsoe College, Cranfield Institute of Technology	01/07/98	31/03/99	7,861
MH0226 (M01006)	Routes of enteric micro-organism contamination of beef and lamb carcasses and improved intervention measures	Silsoe Research Institute	01/07/98	30/06/01	169,313
MH0229 (M01009)	Source & spread of particulate & bacterial contamination between cattle during farm to abattoir phase of production	ADAS Consulting Ltd	01/10/99	30/09/02	51,427
MH0230 (M01010)	Evaluation of deep litter wood chippings for bedding finishing cattle	Scottish Agricultural College, Edinburgh	01/04/99	31/05/99	2,409
MH0231 (M01011)	A survey of factors affecting the MHS scores and bacterial load of sheep arriving at abattoir	ADAS Consulting Ltd	01/01/00	31/12/00	21,225
MH0232 (M01012)	Study of the residues resulting from the use of hyperchlorinated water in poultry washes	Central Science Laboratory, York	01/01/00	30/09/00	65,535
MH0233 (M01013)	Farm management practices to improve the visible and microbiological cleanliness of cattle hides at slaughter	ADAS Consulting Ltd	01/01/00	31/12/02	24,202

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
MH0235 (M01015)	Factors affecting the presence and spread of human bacterial pathogens in sheep	ADAS Consulting Ltd	01/01/00	30/06/03	27,868
TOTAL					465,442

M03 – Transmissible Spongiform Encephalopathy (TSE)

1. Background

It is important to ensure that all animal products meant for the human food chain are free of any potential infectivity of the agents responsible for the transmissible spongiform encephalopathies (TSEs). Whilst we are fully aware that cattle can succumb to Bovine Spongiform Encephalopathy (BSE), and that the development of this disease was in all probability partly due to the feeding of bovine by-products back to cattle, we need to verify that other farm species such as pigs and poultry are not susceptible to similar TSE diseases. In addition it is important to know which tissues of BSE infected cattle carry BSE infectivity.

A number of experiments have been performed to investigate the transmissibility of BSE and scrapie to pigs and chickens by various routes of challenge. Tissues from cattle experimentally challenged with BSE have been bioassayed in mice and cattle to determine the temporal and spatial development of infectivity and pathology of BSE in cattle.

Because of the controls in place, it is unlikely that cattle or sheep infected with TSEs are consumed by human beings. However, research has been carried out to ascertain the risk of contamination of cattle and sheep carcasses with CNS material, both at killing and subsequent butchery. Statistical models have also been developed to determine modes of transmission and the risk to human and animal health.

The costs for these experiments in Financial Year 1999/2000 were c. £2.6 million.

2. Outputs

Attempted transmission of BSE to fowl and pigs

In the initial attempt to transmit BSE to domestic fowl by parenteral inoculation (SE1805) and oral challenge (SE1806) neither group of birds succumbed to BSE nor displayed any signs of TSE infection. Central

nervous system (CNS) tissues from birds in these early experiments were collected and sub-passaged intracerebrally into two further groups of chickens; these latter trials are currently underway.

Mouse bioassay of nervous tissues from birds originally challenged with BSE are complete and no infectivity was detected. A number of birds have died due to intercurrent disease during the sub-passage phase. Histopathological examination of these birds has shown no significant lesions consistent with a TSE. All other birds are clinically normal.

Similar experiments have been carried out with pigs in which the transmissibility of BSE (SE1817, SE1840) and scrapie (SE1822) to pigs by oral challenge has been investigated. Pigs were fed BSE (SE1817) infected bovine brain homogenate and monitored closely for any signs of TSE infection. In addition, scheduled kills at 2 and 7 years post challenge were performed at which time tissues were collected for bioassay in mice.

At 2 years post challenge there was no outward signs of TSE disease in any of the culled pigs. Bioassays of tissue from these pigs have been completed and no infectivity was found in any of the tissues tested. At 7 years post challenge all surviving pigs were killed, and again there was no evidence of TSE on histopathological examination of brain tissue. Bioassays of neural and non-neural tissues from this final kill were completed in May 2000; there was no confirmatory evidence of TSE infectivity in any of the tissues.

A parallel experiment was performed where pigs were challenged orally with brain homogenate from scrapie affected sheep (SE1822). At 2 years post challenge, pigs killed as scheduled were all clinically normal and histopathological examination of CNS material revealed no lesions consistent with TSE. Bioassay of tissues collected from the 2 year cull is complete and no infectivity has been found. Remaining pigs are clinically normal and are scheduled to be killed at 7 years post challenge.

Identification of tissues carrying infectivity in cattle experimentally infected with BSE

To study the progression of BSE in cattle more closely calves were orally exposed to a relatively high dose of BSE infected brain homogenate (SE1901). The challenged cattle were then culled at time intervals of 4 months starting at 2 months post challenge. The final group of cattle was culled at 40 months post-challenge. At necropsy a range of neural and extra-neural tissues were sampled for histological examinations and for infectivity by mouse bioassay.

Evidence of scrapie associated fibrils and vacuolar changes were first observed in cattle killed 32 months post challenge and unequivocal clinical signs became apparent in one animal 35 months post challenge. Infectivity

was detected in the frontal cortex of the brain from 38 months post challenge; the caudal medulla, spinal cord and dorsal root ganglion from 32 months post challenge; the trigeminal ganglion from 36 months post challenge and a low level of infectivity was detected in bone marrow at 38 months post challenge only. The distal ileum displayed infectivity from 6 to 18 months post challenge and again from 36 months. The early infectivity may be the result of the experimental conditions, as a large oral dose of infectivity was given to the cattle.

In an attempt to increase the sensitivity of the experiment some of the same tissues already inoculated into mice were used to repeat the bioassays in cattle (SE1824, SE1825). To date, in the calf bioassay, three tissue groups have been found to contain infectivity; CNS material collected from cattle 32 months post challenge, and distal ileum collected from 10 and 18 months post challenge. The same samples were found to contain infectivity in the original mouse bioassays. All cattle challenged with other tissue groups remain clinically normal.

Studies in abattoirs

In order to investigate the majority of stunning methods used in UK abattoirs, cattle were stunned with penetrating and non-penetrating captive bolt guns, with and without air injection and with or without pithing (SE11831). The level of resultant CNS material in the blood collected from the jugular vein was measured.

It was shown that the use of pneumatically operated guns at slaughter (those in which air is injected into the cranial cavity with no subsequent pithing) increase the risk of CNS dissemination into the lung. Pithing was also shown to increase this risk, even when following non-pneumatic stunning. The extent to which CNS material could contaminate the carcass, from its point of measurement in the jugular veins, was not determined. A similar study is underway for the slaughter of sheep (SE1832).

Techniques for measuring possible CNS dissemination during the splitting of cattle carcasses have been developed (SE1826) and will be applied to a wider study of carcass contamination in abattoirs (SE1838) Cattle carcasses are being examined for CNS contamination following splitting with conventional band saws and reciprocating saws.

Novel techniques to avoid possible contamination have been investigated with the result that one new type of saw is progressing towards commercial development (SE1826).

Statistical models of TSE transmission

Statistical models have been produced for feed borne infection that considers the risk for an animal of exposure to infected feed (SE0219).

A model where risk is proportional to the amount of infected feed consumed by a herd has been found best to reflect existing data. Geographical clustering of cases of BSE in herds of cattle has been described by data, although the time of onset of the epidemic was fairly conserved. A model to describe the dynamics of scrapie transmission is in development.

Risks to human health from the ruminant TSEs have also been assessed and found difficult to pin down due to key biological uncertainties.

3. Disseminating Activity

Results from these projects have been reported in peer-reviewed journals. Some of these results were presented at an international conference in Tübingen, Germany, which was attended by most of the main workers in the field of TSE research. The work on the epidemiology has been widely presented at meetings at Universities and other institutions and at international conferences and workshops. The Spongiform Encephalopathy Advisory Committee is kept up to date with interim results from these projects. Results from these projects are also used to inform consultation exercises at the World Health Organisation and Office Internationale des Epizooties.

4. Future Activity

The study of techniques used in abattoirs will be widened to a survey of selected abattoirs across the EU, with half funding from the EU Commission. Carcasses in a selection of abattoirs in the UK will be investigated for possible contamination with CNS using the techniques developed in this programme.

M03 – Transmissible Spongiform Encephalopathy (TSE)

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
SE0219 (M03001)	The epidemiology of TSEs in ruminants and assessment of possible associated risk to human health	University of Oxford	01/01/97	30/09/00	81,115
SE1805 (M03002)	Transmissibility of BSE to domestic fowl by injection with brain homogenate	Veterinary Laboratories Agency	01/04/92	31/03/02	4,752

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
SE1806 (M03003)	Transmissibility of BSE to domestic fowl by oral exposure to brain homogenate	Veterinary Laboratories Agency	01/04/92	31/03/02	4,752
SE1822 (M03005)	Transmissibility of scrapie to pigs by oral exposure to brain homogenate	Veterinary Laboratories Agency	01/04/95	31/03/05	35,484
SE1824 (M03006)	Bioassay of BSE infectivity in non-neural tissues by intracerebral inoculation of cattle	Veterinary Laboratories Agency	30/06/96	31/03/13	1,009,649
SE1825 (M03007)	Bioassay of BSE infectivity in non-neural tissues by intracerebral inoculation of cattle	Veterinary Laboratories Agency	01/04/98	31/03/14	1,033,496
SE1826 (M03008)	Measures to reduce contamination of meat & environment with CNS tissue during slaughter & processing of cattle & sheep	Silsoe Research Institute	01/02/98	31/01/01	107,624
SE1838 (M03009)	Pilot survey of cattle and sheep slaughter, dressing and butchery practices in use within the UK	Silsoe Research Institute	01/09/99	31/12/99	4,690
SE1840 (M03010)	Further studies on the transmissibility of BSE to pigs	Veterinary Laboratories Agency	01/07/99	30/06/06	91,216
SE1901 (M03011)	Pathogenesis of experimental BSE in cattle	Veterinary Laboratories Agency	01/04/92	31/03/01	195,956
SE1817 (M03014)	Transmissibility of BSE to pigs by oral exposure to brain homogenate	Veterinary Laboratories Agency	01/04/95	31/03/01	26,486
Total					2,595,220

Radiological Protection (MINIM PP1:07)

RP01 (now R01) – Assessment of the effects of radioactivity in the environment

1. Background

The Food Standards Agency is responsible for protecting the food supply from undue or unacceptable contamination with radioactivity. To discharge these responsibilities the FSA needs to be able to assess the potential impact of planned and accidental discharges of radioactivity on the foodchain and on radiological doses to consumers. The Environment Agency and the Scottish Environment Protection Agency authorise the discharge and disposal of radioactive waste from both licensed nuclear sites and other users of radioactivity in England, Wales and Scotland and are required by statute to consult the Food Standards Agency on the determination of authorised limits.

This programme is concerned with the assessment of the impact of discharges of radioactivity to the atmosphere or geosphere on terrestrial foodstuffs and to the hydrosphere and their impact on aquatic foodstuffs. Assessment of the impact of naturally occurring radionuclides and routine and emergency discharges are covered; research relevant to all other aspects of emergencies is in a separate programme.

The specific objectives of the programme are:

- To provide assessment methods for nuclear discharges and environmental activity which are:
 - comprehensive (accommodate all scenarios that will need to be modelled);
 - forward looking (anticipate future needs and identify pathways and routes of exposure that might become important or which are not adequately studied at present);
 - functional;
 - reliable and produce results (i.e. necessary accuracy and precision) that the users can have confidence in and be accepted by the assessment community, and have public trust;
 - be able to express results in a way that aids the decision making process.

Most of the cost of this work will be recovered from the industry by charges levied under the provisions of the Environment Act 1995.

The cost of the programme in the 1999/00 financial year was ca. £568k.

2. Outputs

Initial development of a probabilistic terrestrial food chain model has been completed. This will enable the FSA to calculate the distribution of doses to humans from ingestion of contaminated foods taking into account the uncertainty in many of the parameters which govern the accumulation of radionuclides in the foodchain. Suitable input parameters are being derived for UK agricultural conditions in a separate project.

A revised method has been developed for performing screening assessments of discharges to sewers and watercourses from small users of radioactivity. This gives the FSA much greater flexibility in applying site specific data to such assessments and also incorporates previously excluded pathways such as the application of sewage sludge to agricultural land.

An investigation to determine whether activity concentrations in marine materials are affected by the temporal profile of the discharges demonstrated that the FSA's predictions of activity concentrations in marine organisms remain valid regardless of the time-scale of the discharges.

The FSA's modelling capabilities have been refined through a review of literature relevant to the modelling of ^{35}S , ^{14}C and ^3H in animals. A further project has been completed which explored the relationship between historical environmental monitoring data and discharge data from individual UK sites which can be used to support predictive environmental modelling.

A programme of habit surveys in the vicinity of nuclear sites has continued during 1999/2000 and has continued to provide valuable information for the realistic treatment of critical group behaviour.

During the last year the FSA has been an active participant at the International Atomic Energy Agency (IAEA) BIOMASS programme (Biosphere Modelling and Assessment methods). It took part in the Tritium Working Group and is currently participating in the Fruit Working Group, which it sponsors, and the Dose Reconstruction Working Group. The main activity of these groups is to test and validate models for the transfer of radionuclides in the environment. A comprehensive review has been prepared by the Fruit Working Group for publication as an IAEA Working Document. Parts of this review have been accepted for publication as a special issue of the Journal of Environmental Radioactivity. Model

intercomparison and validation exercises have been successfully completed. The FSA and its contractors also participate in BIOMASS Theme 1, Radioactive Waste Disposal, whose objective is to develop the concept of reference biospheres into a practical system for application to the assessment of the long term safety of radioactive waste repositories.

3. Dissemination Activity

Progress and output from individual projects in the programme was discussed during February 2000 at a research seminar organised by Radiological Safety Division. Participants included contractors, academics, and representatives from the nuclear industry and officials from a number of Government Departments and Agencies.

Final project reports are lodged in the in-house library and made available on request. A number of papers based on projects in this programme have been published in the open literature.

4. Future Activity

The main thrust of the programme is to further improve the FSA's capability to assess the foodchain implications of radioactive waste disposals. Much of the work described in this document is continuing, the information gathered will be used in updating the assessment models and general methodology and widening the scope of model applications. This includes work considering the variability of critical group doses and the implications for the control of radionuclide releases.

The FSA's capability in assessing the impact of proposed discharges from small users of radioactivity to sewers and watercourses will be improved by a project which will provide a computer based toolkit to assess the combined impact of discharges from many individual sites. This will be supported by a project which will derive hydrographic data related to river networks and the operation of sewage works in the UK.

Work has been commissioned on providing a probabilistic modelling capability for the aquatic environment to mirror the capability provided by the probabilistic terrestrial food-chain model. This work will be complemented by a project which will quantify uncertainties in aquatic assessments.

Gaps in the FSA's ability to assess discharges will be addressed with projects to investigate the behaviour of ³⁶Cl in the environment and the accumulation of radionuclides in fodder and oilseed crops. In addition, existing food chain models will be refined through a project which will study the effect of plant growth on the interception of nuclides and the compilation of a database on crop growth characteristics.

5. Comments

The Programme Manager is Dr Paul Naylor of the Radiological Safety Unit.

RP01 (now R01) – Assessment of the effects of radioactivity in the environment

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
RP0151 (R01001)	Development of an international radionuclide flux database	Mouchel Consulting Ltd	01/09/96	21/07/00	12,485
RP0152	Habits surveys: terrestrial foods and sources	Centre for Environment, Fisheries and Aquaculture Science	01/04/96	31/03/99	12,475
RP0157 (R01004)	Modelling and experimental study on the transfer of deposited radioactivity to fruit	Mouchel Consulting Ltd	01/05/96	30/11/99	29,474
RP0158	Parameters and sub-models for dry deposition of particulate contaminants to shrubs and fruit trees	CARE	01/05/96	30/04/99	23,281
RP0160 (R01006)	Deposition of gaseous H-3, C-14, S-35 to fruit	CARE	01/10/96	31/01/00	12,860
RP0162 (R01008)	Parameters and sub-models for wet deposition of soluble and particulate contamination to crops	CARE	01/09/96	31/08/99	31,290
RP0164 (R01009)	Development of a model for the prediction of S-35 content in crops	CARE	01/10/97	30/09/00	35,596
RP0169 (R01010)	Proposal to facilitate probabilistic modelling in MAFF foodchain models	QuantiSci Ltd	01/04/98	31/12/99	35,850
RP0170 (R01011)	Measurement of I-129 in fruit	Westlakes Research Ltd	01/04/98	31/03/01	13,003
RP0171 (R01012)	Site-specific data for modelling aquatic environments. Option3	Centre for Environment, Fisheries and Aquaculture Science	01/04/98	31/03/00	19,290
RP0172 (R01013)	Technical Secretariat for BIOMASS Theme 3 working group on radionuclide transfer	QuantiSci Ltd	01/03/98	31/10/01	11,084

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
RP0173	Technical support for MAFF in assessments of the foodchain impact of disposal of solid radioactive waste in shallow or deep repositories	Mouchel Consulting Ltd	01/04/98	31/03/99	2,500
RP0178	Peer review of MAFF aquatic model code IDLE 11	QuantiSci Ltd	01/04/98	31/12/98	5,953
RP0179 (R01020)	The use of phylogeny to simplify predictions of radionuclide concentrations in crop plants	University of West of England	01/01/99	31/12/00	51,909
RP0180 (R01021)	Use of monitoring data in environmental modelling	Centre for Environment, Fisheries and Aquaculture Science	01/04/99	31/03/00	17,063
RP0181 (R01022)	An investigation of time varying discharges of radioactivity to the marine environment	Centre for Environment, Fisheries and Aquaculture Science	01/04/99	31/03/00	13,425
RP0182 (R01023)	Organic forms of tritium in foodchains	Centre for Environment, Fisheries and Aquaculture Science	01/06/99	31/05/01	25,605
RP0183 (R01024)	Habit surveys: Terrestrial foods and sources 11	Centre for Environment, Fisheries and Aquaculture Science	01/06/99	31/05/02	28,489
RP0184 (R01025)	Transfers of radionuclides deposited onto the external surface of plants, particularly fruits to internal compartments	ADAS Consulting Ltd	01/04/99	31/03/02	30,679
RP0185 (R01043)	Technical support for MAFF in assessments of the foodchain impact of disposal of solid radioactive waste in shallow or deep repositories	Mouchel Consulting Ltd	09/04/99	31/03/00	8,500
RP0186 (R01027)	Derivation of Ra-226 parameters for SPADE	WS Atkins	06/04/99	15/03/00	6,880

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
RP0187 (R01028)	Expert elicitation of modelling parameters explicitly relevant to UK environmental and agricultural conditions	AEA Technology	01/04/99	31/03/01	23,750
RP0188 (R01029)	Review of data suitable for foodchain modelling of C-14, H-3 and S-35 in animals	AEA Technology	01/06/99	31/03/00	17,920
RP0189 (R01030)	Examination of carbon-14 methane in atmospheric air in the locality of the Drigg trenches	Radio Carbon Dating	01/04/99	31/03/00	14,940
RP0190 (R01031)	Accumulation of 99Tc in the Irish Sea	Centre for Environment, Fisheries and Aquaculture Science	01/04/99	31/03/01	67,531
RP0191 (R01032)	Measurement of tritium speciation around the Nycomed Amersham site at Cardiff	Radio Carbon Dating	01/09/99	31/06/00	5,700
RP0192 (R01033)	The variability in critical group doses and implications for control of radionuclides releases	National Radiological Protection Board	01/09/99	31/08/00	1,470
RP0193 (R01034)	Assessment of organically bound tritium (OBT) dispersion and accumulation in the environment	Southampton University	28/10/99	27/10/02	8,526
TOTAL					567,528

RP02 (now C05) – Food contamination emergencies

1. Background

The Food Standards Agency has a statutory responsibility for protection of the food supply from accidental contamination by radioactive materials or toxic chemicals. Contamination of food may be direct or occur via an indirect route such as animal feeding stuffs. Risk assessments are made to judge the potential for contamination of the food chain. If deemed necessary the Food Standards Agency can place restrictions on foods following an accident by use of either the Food and Environment Protection Act 1985 (FEPA) or Section 13 of the Food Safety Act 1990. Statutory powers for the

control of food can be extended to animal feeding stuffs. The primary objective of this programme is to develop the capability of the Food Standards Agency to respond rapidly and effectively to emergencies where accidental release of toxic chemicals and/or radioactive materials may affect the safety of the food chain. The scale and type of these incidents can vary greatly but certain attributes of effective response are always necessary, including the ability to rapidly gather and assess information, to quickly assess the hazard and to take appropriate steps to protect public health. This programme seeks to improve the Agency's knowledge, tools and skills in these areas. The cost of the programme in the 1999/2000 Financial Year was ca. £228k.

2. Outputs

Development is continuing on a mobile emergency monitoring facility for the rapid analysis of potentially contaminated samples in the event of a nuclear incident (RP0258). Work is also continuing on an investigation of statistical techniques for the estimation and characterisation of contaminated areas (RP0242). A new project is evaluating both aerial and ground based monitoring techniques and will identify the most appropriate combination of these methods for different types of nuclear accidents (RP0260). This project is jointly funded with the Environment Agency. A project has calculated nuclear emergency planning zones, which represent areas that could potentially be affected by precautionary advice or food restrictions following an accident at a nuclear establishment. The output of this will assist rapid decision making and dispersion model interpretation during an emergency (RP0254).

A comparison of the Food Standards Agency's emergency response atmospheric dispersion model is being made with other similar models to ensure that the Agency's approach remains appropriate (RP0261).

A project, jointly funded with the Environment Agency, is looking at potential nuclear accident scenarios that could affect marine food pathways and is calculating the resulting activity concentrations in sediments, fish, molluscs and crustacea. The dose from critical group consumption of these foods is also being calculated (RP0257).

Following a nuclear accident the amount of food which is waste because it is contaminated in excess of the EC Community Food Intervention Levels could be considerable. A project has examined different methods for the disposal of this food with particular emphasis on the difficult issue of milk disposal (RP0256).

A review has been completed of the Agency's emergency arrangements for responding to nuclear weapons accidents and will examine how well current

procedures work. Any gaps in knowledge or information available will be identified together with any inconsistencies in approach (RP0255).

The training needs of emergency response staff in the Food Standards Agency were examined. Training programmes were then recommended, taking account of the differing needs of staff who perform a variety of roles (RP0259).

3. Dissemination Activity

The Radiological Safety Unit presents results and progress from individual projects in this programme at its annual research seminar. Participants at these include contractors, academics, representatives from the nuclear industry and officials from a number of Government Departments and Agencies. Final reports are made available from the in-house library and publicised in the Food Surveillance Information Bulletin. Results are also distributed to other committees and Working Groups as appropriate. Contractors are encouraged to present results of their research at scientific conferences both in the UK and worldwide

4. Future Activity

Following a review of the programme in March 1998 the ROAME A was revised to reflect the increased responsibility for emergencies involving chemical contamination. The requirement for research relating to chemical emergencies is already addressed in the programme and future work will continue on identifying the potential impact of likely contamination events. The future emphasis of the programme will also continue to be on developing the Agency's response capability through, for example, streamlining emergency response procedures.

5. Comments

The Programme Manager is Dr Paul Naylor of the Food Standards Agency's Radiological Safety Unit.

RP02 (now C05): Food contamination emergencies

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
RP0242 (C05001)	Investigation of measurement based statistical estimation and characterisation techniques for use during accident response	National Radiological Protection Board	01/11/96	31/10/00	36,118
RP0244 (C05003)	Spatial analysis of vulnerable ecosystems in Europe (SAVE) spatial and dynamic prediction of radionuclide fluxes into European foods	ITE	01/04/96	31/03/00	20,000
RP0251	Implementation of a large-scale emergency exercise to test MAFF response to contamination of the food chain following a chemical accident (POLAX)	Det. Norske Veritas Ltd.	06/04/98	05/04/99	4,504
RP0252	Review of human foodchain consequences of chemical accidents	ITE	01/04/98	30/11/99	27,021
RP0254	Nuclear accidents and emergency planing zones	AEA Technology	01/04/98	31/03/99	9,598
RP0255	Nuclear weapons accidents: Review of MAFF's emergency arrangements	National Radiological Protection Board	01/02/99	31/01/00	12,411
RP0256 (C05012)	Management options for food production systems affected by a nuclear accident	National Radiological Protection Board	01/10/98	31/03/01	20,475
RP0257 (C05013)	Scoping assessments for accidental releases of radioactivity to marine environments	AEA Technology	01/04/99	31/03/00	7,500
RP0258 (C05014)	Development of a mobile emergency monitoring facility	Centre for Environment, Fisheries and Aquaculture Science	01/04/99	31/03/03	27,736

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
RP0259 (C05015)	Training needs analysis of MAFF's human resources for emergency response and development of a training programme	National Radiological Protection Board	01/06/99	31/10/99	13,550
RP0260 (C05016)	Optimum combination of aerial and ground-based monitoring for different types of nuclear accidents	AEA Technology	01/04/99	31/03/01	21,700
RP0261 (C05017)	Comparison of atmospheric dispersion models for emergency response assessment within JFSSG	AEA Technology	01/08/99	30/09/99	6,705
RP0262	Investigation of spatial and temporal aspects of gamma spectrometry in north-west England	SURRC	01/03/99	31/03/01	20,000
TOTAL					227,318

RP03 (now R02) – Research in support of radiological surveillance

1. Background

In order to protect consumers from undue or unacceptable radioactive contamination an extensive surveillance programme for radionuclides in the terrestrial and aquatic foodchains is carried out by the FSA. This provides independent monitoring of the levels of activity found in foodstuffs.

This programme supports this work; ensures there are no unidentified significant pathways or routes of exposure to the consumer; and maximises the quality of analytical methods used to ensure that methods are reliable and precise, whilst being cost effective. It also aims to maintain the FSA's authority and requirements in radiological surveillance nationally and internationally, and provide flexibility to accommodate changing requirements. Lastly, it includes investigations into areas still affected by sheep controls under the Food and Environment Protection Act 1985 because of the Chernobyl accident, with the aim of lifting restrictions when consistent with food safety.

Laboratory analytical methods should provide the FSA with results of adequate sensitivity to assess doses to the consumer accurately and determine that national and international dose limits are not exceeded,

whilst being as cost effective as possible. Research supports the development of new methodologies for the surveillance programme where needed and the identification of the most appropriate method of analysis where more than one method is available. It also reviews the quality of the current analytical methods by arranging intercomparison exercises to assess individual laboratory performance.

The surveillance programme needs to be directed towards the most appropriate pathways to allow quantification of the most likely highest doses to consumers. Dietary studies of consumers living around nuclear sites are commissioned to assess local food intake and allow doses to be calculated to high-rate consumers and critical groups. The radiological significance of all foodchain pathways is kept under constant review in order to detect minor or sub-critical routes of exposure, new pathways or those that may be present infrequently or affect only a few people.

During the 1999–2000 financial year, the programme consisted of nine projects, placed with six different contractors and £180K was spent. Two new projects were introduced to the programme in the year, five projects were completed and four were continued into 2000–2001. A mid-term review of the programme was carried out and new ROAME A document produced in 1998.

2. Outputs

Duplicate diet study

This year, duplicate diet studies were carried out around the Hinkley Point and Sellafield sites. For the study local to the Hinkley Point power stations, the maximum sample activity found was 78 becquerels per kilogram for both H-3 and C-14, lower than those recorded in RIFE for 1998, and the total annual mean doses were <8 microsieverts (μSv) for adults and <13 μSv for children with C-14 contributing between 87 and 92% of the total dose in individual diets. The mean doses for the study group were lower than those for the control group. These results confirm that the doses assessed for the Hinkley Point area in the RIFE report are realistic and not overly conservative. The Sellafield study is scheduled to report in July 2000. These doses are negligible compared with the average annual UK background radiation dose of approximately 2,200 μSv received predominately from sources of natural radioactivity.

Seasonal variations in crabs and lobsters

A pilot study to confirm that key radionuclides are measurable by specified analytical techniques in crabs and lobsters caught off the Calf of the Isle of Man has been completed and the principal 12 month sampling campaign started in February 2000. The report is due in July 2001.

Laboratory intercomparison for tritium (H-3) in fish and milk

This project is in response to concerns over high levels of tritium found in fish in the Bristol Channel, thought to be due to its chemical form. Analytical methods for Organically Bound Tritium (OBT) in foodstuffs differ between laboratories and this project is designed to compare their accuracy and reliability. The report is due in July 2000.

Quality control in the measurement of radionuclides

The quality control project for the determination of radionuclides in foodstuffs was completed and a final report received in 1999. This intercomparison project showed good agreement between the results of “expert laboratories” and significant variations between “non expert” laboratories, particularly at low levels of spike nuclides whose analysis required radiochemical separation prior to counting. This work has given the FSA greater confidence in the results supplied by its main radioanalytical contractors.

Continuation of a quality control programme for the measurement of radionuclides

This continuation project is designed to further test the radioanalytical abilities of laboratories using spiked foodstuffs. Spiked samples have been sent out to participating laboratories. The report is due later this year.

Optimisation tool for monitoring programme

The development of a computer-based decision-aiding tool, to aid in prioritising the requirements for surveillance programmes to optimise use of resources, has been completed. Work will now be progressed internally to populate the associated databases with actual data. When complete, this will enable a better understanding for the basis of the decisions reached concerning the content of our terrestrial surveillance programmes.

Boli to reduce radiocaesium uptake in lambs

In order to explore possible post Chernobyl management options, two projects investigated the feasibility of using boli on lambs. These are designed to reside in the rumen and adsorb radiocaesium in ingested herbage from the gut so reducing levels in sheepmeat. Each project used different formulations and complexing agents to adsorb radiocaesium and the boli were designed to be sufficiently small to be suitable for treating lambs. One project used a widely available fungal by-product of the carbonated drinks industry to produce a vitrified bolus; though this formulation proved ineffective, breaking down either too readily or insufficiently. The other adapted Norwegian wax boli to contain a larger proportion of Prussian Blue in a smaller bolus. Trials indicated this bolus was effective in reducing radiocaesium levels to less than 60% of the control group over 5 to 12 weeks and it could have potential for practical application under appropriate conditions.

Improved analytical techniques for Cerium-144

A more sensitive method than gamma spectrometry for analysing cerium-144 in milk was developed by using ion chromatography in conjunction with Cerenkov counting of the praesodymium-144 daughter. This resulted in an improvement of the limit of detection to 0.025 to 0.05 Bq/l, depending on sample size, enabling more accurate dose estimates to be calculated.

3. Dissemination Activity

Along with other radiological protection research, the results and progress in this programme have been reported at an annual seminar attended by contractors, other regulatory bodies, Government Departments and scientific advisors in February 2000. Final reports of projects are available for dissemination and will appear in published papers. Lists of reports are made available to inter-departmental research working groups attended by representatives of the nuclear industry and are published on the Food Standards Agency's internet site. Copies of all final reports are available for viewing in the library in Nobel House, London. The titles of recently completed projects and details of how to contact the relevant project officer are included in the Food Safety Information Bulletin.

4. Future Activities

An investigation of the methods available for the practical utilisation of environmental measurement data below the limit of detection has been extended and is due to report later this year.

Other projects due to start this year are one to investigate radioactivity in uncommon seafoods, and another to develop a method for chlorine-36 determination in foods using Accelerator Mass Spectrometry (AMS).

5. Comments

The Programme Manager for 1999/2000 was Mrs Caroline Morris of the Radiological Safety Unit, Branch D.

RP03 (now R02) – Research in support of radiological surveillance

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
RP0317 (R02001)	Dietary study to assess intake of radionuclides of people living close to nuclear installations	British Market Research Bureau Ltd	01/04/95	31/07/00	110,689
RP0320 (R02004)	Optimisation of FSA's monitoring programme: Development of a computer-based decision aiding tool	National Radiological Protection Board	01/09/95	30/06/99	2,000
RP0321 (R02005)	The development of a controlled release lamb bolus for the prevention of radiocaesium uptake into the lamb's body	University of Leeds	01/04/97	30/11/99	21,003
RP0322 (N/A)	The development and testing of rumen dwelling AFCF delivery devices suitable for upland lambs	ITE	01/04/97	31/01/99	2,817
RP0324 (R02008)	Investigation of the methods available for the practical utilisation of censored environmental measurement data	National Radiological Protection Board	01/06/98	30/03/00	7,556
RP0325 (N/A)	Improved analytical techniques for Ce-144	AEA Technology	01/04/98	30/04/99	3,392
RP0326 (R02010)	Continuation of a quality control programme for the measurement of radioactivity in foodstuffs	AEA Technology	01/03/98	31/03/00	3,687
RP0327 (R02011)	Seasonal variations in radionuclide concentrations in crabs and lobsters	University of Liverpool	01/06/99	31/07/01	21,824
RP0328 (R02012)	Assessment of UK laboratory performance: Tritium in fish and milk	AEA Technology	01/09/99	31/07/00	7,128
TOTAL					180,096

Animal Feedingstuffs (MINIM PP1:09)

CS01 (now F01) – Feedingstuffs composition and contamination

1. Background

This programme supported those parts of MAFF's and JFSSG's business plans and objectives relating to the composition and labelling of animal feeds. The primary aim is to determine any risks to animal and public health, although the programme also funds research relating to purchasers' choice of feeding materials and confidence in their contents. The effects on animals and the food chain of feeding materials derived from genetically-modified crops (e.g. soya and maize) will be addressed.

During 1999-2000 research on animal feedingstuffs consisted of two projects; one of which was completed and cost £ 20,133 during the financial year 1999/2000. A second project, which started in 1998, had a cost of £67,498 in 1999/2000.

2. Outputs

A project into the effect of processing during the manufacture of animal feeds on DNA integrity was funded in 1999/2000. This project has built on the results obtained from a previous project funded by JFSSG in 1998/1999.

A particular concern is related to feed derived from genetically-modified (GM) crops. Such plants sometimes contain antibiotic resistance genes (used as markers in the development of the strains), which might have some potential for transfer to micro-organisms present in the gastrointestinal tract of livestock. This is considered by some to be a potential route to the development of new strains of antibiotic-resistant bacteria.

The researchers have developed methods to help determine whether processed feed materials contain 'gene-size' fragments of DNA.

The final project report provides a) information on the development of the DNA size determination method; and b) results from the use of this method on a variety of feed materials.

The second project was commissioned in 1998 to help develop official methods for the control of probiotic additives in animal feeds. The part of the project funded by JFSSG aims to develop methods for the enumeration, isolation, identification and characterisation of yeasts used as feed additives.

Other partners in this EU shared cost project are funding similar work into the various types of bacteria used (or potentially used) in probiotics products. It is expected that the result for the whole project will be used to help support enforcement of Directive 70/524/EEC (feed additives Directive), and will be incorporated into statutory methods used by Official Laboratories. The project is expected to continue until the end of 2001; there are yet to be any outputs.

3. Dissemination Activity

A copy of the final report for the project concerned with the effect of the commercial processing of feed on the integrity of DNA was placed in the Nobel House library recently for public inspection. The library staff can provide copies of the report for a nominal charge.

The researchers wrote and presented a paper to the Nottingham Feed Manufacturers' Conference 2000.

It is expected that the researchers will wish to write an additional paper for an appropriate refereed journal.

4. Future Activity

It is likely that the newly-formed Advisory Committee on Animal Feeds (ACAF) will wish to review the work of this programme.

CS01 (now F01) – Feedingstuffs composition and contamination

Project Code (FSA Code)	Project Title	Contractor	Start Date	End Date	Cost in FY 99/00 £
CS0117 (F01001)	Methods for the official control of probiotics used as feed additives	Central Science Laboratory, York	01/10/98	30/09/01	67,498
CS0118 (F01002)	The effects of commercial scale processing on the integrity of DNA in animal feeds	ADAS Consulting Ltd	01/03/99	30/04/00	20,133
TOTAL					87,631

Glossary of Terms

AMS	Accelerator mass spectrometry
BBSRC	Biotechnology and Biological Scientific Research Council
BSE	Bovine spongiform encephalopathy
BSI	British Standards Institute
COMA	Committee on Medical Aspects of Food and Nutrition Policy
CVD	Cardiovascular disease
DH	Department of Health
DNA	Deoxyribonucleic acid
EC	European Community
ELISA	Enzyme-linked immunosorbent assay
EPIC	European Prospective Investigation on Cancer
ER	Oestrogen receptor
EU	European Union
FFP	Fitness for purpose
FOR A	Food risk assessment
FSA	Food Standards Agency
FY	Financial year
GC-MS	Gas chromatography-mass spectrometry
GMO	Genetically modified organism
GMP	Good manufacturing practice
HACCP	Hazard analysis and critical control point
HDL	High density lipoprotein
HQ	Head Quarters
i.e.	id est – That is to say
IGFBP-1	Insulin-like growth factor-1 binding protein
<i>In vitro</i>	Occurring outside the living organism
<i>In vivo</i>	Occurring in the living organism
JFSSG	Joint Food Safety and Standards Group
LC	Liquid chromatography
MAFF	Ministry of Agriculture Fisheries and Food
MRC	Medical Research council
MUFA	Monounsaturated fatty acids
NDNS	National Diet and Nutrition Surveys
NSP	Non-starch polysaccharides
PAHs	Polynuclear aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
PCR	Polymerase chain reaction
PG	Prostaglandin
PUFA	Polyunsaturated fatty acids
SCFA	Short chain fatty acids
SFA	Saturated fatty acids

