Food Institutional Research Measure

Food For Health Research Initiative (FHRI)

Final Report

'Safe and Healthy Foods'

DAFM Project Ref No: 07FHRITAFRC5
Start date: 31/12/2007
End date: 31/12/2013

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Prof. Mike Gibney, University College Dublin
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Prof. Francis Butler, University College Dublin
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Dr Geraldine Duffy, Teagasc Food Research Centre Ashtown
Prof. David McDowell, University of Ulster, Jordanstown.

Please tick below the appropriate area on the research continuum where you feel this project fits

BASIC/FUNDAMENTAL | APPLIED/PRE COMMERCIAL

X

Key words: Food; Contaminants; Databases; Risk
1. **Rationale for Undertaking the Research**

The research project encompassed three thematic areas of work, namely, Chemical Contaminants, Nutrition and Health and Biological Contaminants.

The chemical contaminants sub-project set out to address a number of gaps in the area by providing new tools and knowledge for regulatory agencies and the greater scientific community. It also set out to develop expertise in new areas of research in the participating research institutes. A major sub-theme of this sub-project was to develop tools for risk based surveillance purposes, which is an emerging topic in food safety. The project provided a solution to tracking the usage of veterinary drugs and feed additives by developing a new veterinary drug database. In addition, Ireland's National Food Residue Database was to be kept current by uploading the latest residue surveillance data. Reports from both of these databases were employed to support the development of risk ranking systems for veterinary drugs and feed additive residues. This risk ranking list was to be employed by regulatory agencies and industry to prioritise the monitoring and surveillance activities. Secondly, the project complemented and supported existing research being carried out as part of the National Residue Control Plan by developing analytical tests that addressed a number of gaps. These included the development of comprehensive tests for anticoccidials, illegal dyes and antibiotics (including the difficult to analyse aminoglycosides). The project also embarked on new areas of research including the pyrrolizidine alkaloid plant toxins and sex hormones. Furthermore, it applied these new tests and existing tests to provide comprehensive data for knowledge generation and risk assessment purposes.

The nutrition and health sub-project proposed to develop and extend the nutrition databases in the areas of food ingredients and food consumption. This was achieved through maintaining and expanding the Irish National Food Ingredient Database (INFID) and the Irish Supplemental Food Composition Database to provide critical data on food ingredients and food consumption. This updated data for adults and introduced new data for infants for the first time. This sub-project is an integral part of a multi-disciplinary surveillance system, designed to complement activities in other areas of food safety and nutrition. It builds on previously FIRM funded initiatives which established databases in both food composition and food ingredients, both of which have proven to be invaluable to the national regulatory authorities in addressing risk assessment issues in the past.

The biological contaminants research team set out to develop a unified microbial database to collect data from the numerous stakeholders on the island. At the commencement of the project, data was generally managed through Laboratory Information Management Systems (LIMS) and transmitted in reports directly to FSAI. Although a considerable amount of data is being generated, risk analysis was not usually performed. A major bottleneck in implementing the risk based management strategies is the fact that often the data was not available in formats appropriate to quantitative risk assessment. In many cases the data was only available in summarised reports whereas many risk assessment applications require access to databases containing the raw information set so that appropriate estimates of, for example, prevalence of a particular pathogen at a point in the food chain can be properly estimated.
2. **Research Approach**

A core research group was formed from the major chemical contaminant research teams on the island, namely, Teagasc Food Research Centre Ashtown, Cork Institute of Technology, Agri-Food and Biosciences Institute, University College Dublin and Queens University Belfast. Novel methodologies were developed on the project for targeted detection of chemical contaminants in food using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). In addition, non-targeted approaches were applied to identify novel contaminants including receptor based assays and high resolution mass spectrometry systems at QUB. In some cases, where targeted LC-MS/MS methods could not be developed, multiplex immunoassay screening methods were validated and employed for aminoglycosides and some coccidiostat residues. During the early course of the project, it was found that the development of VetFAD needed dedicated staffing and additional resources were dedicated for this task.

Two food consumption studies were conducted to update INFID for adults and also for toddlers. This involved the collection of packaging material, shipment to a central base where new packaging material will be set aside for food contact material analyses. The packaging material labels were examined to update INFID. On pack data, food manufacture data and direct analysis were employed to update the packaging database. Similarly the Supplemental Food Composition Database for adults was extended to include foods for infants and data on botanical and herbal products. This data was collected from a new national adult food consumption study (n 1,500) that commenced in 2008 and a food consumption study of infants aged 1-4 years that started in 2009. Data came from a number of sources, including analysis for selected products, manufacturers’ data and data published in the literature.

The Central Microbial Database was developed by UCD in collaboration with Central Veterinary Research-DAFM, FSAI, Teagasc and UUJ. The database incorporates data for major pathogens in Irish food generated by the major regulatory and research institutions in Ireland. The data was validated through the uploading of three years of data from FSAI and DAFM. Novel features of the project involved, additional work on a national database of sub-typed isolates (by PFGE to PulseNet standard and serotyping) of three relevant food poisoning organisms. This was the first time such a database has been developed in Ireland. The database was populated with strains of Listeria monocytogenes, Salmonella isolates and VTEC O157 isolate data.

3. **Research Achievements**

**Sub-project 1: Chemical contaminants**

*New Database on Veterinary Drugs Sales Usage*

A unique veterinary drug database (VetFAD) was developed for the first time containing sales data on veterinary drug products sold on the island between the years 2008 and 2013. VetFAD was populated with information from veterinary drug product label data from the IMB website and the with population data for different food producing species.
A series of VetFAD reports were developed that provide estimates of the number of doses given to different animals and tonnage of active ingredients.

**New analytical tests of banned substances and licensed veterinary drugs in food**

- A new method chemical test based on ultra-high pressure liquid chromatography with tandem mass spectrometry detection (UHPLC-MS/MS) for four nitrofuran residues in honey.
- A multiplex immunochemical screening method was developed to detect four nitrofuran residues in honey. This has potential for use in non-expert laboratories.
- A new test was developed for the determination of dyes in aquaculture and poultry meat samples using UHPLC-MS/MS.
- A new method was developed and validated by Teagasc to detect eight nitromidazoles in aquaculture and poultry meat samples.
- A multi-residue UHPLC-MS/MS method was initially developed to measure 23 coccidiostat residues in egg, milk and muscle. The method is a significant improvement of other analytical methods based on the range of analytes covered and validation work completed.
- A screening assay was developed for the simultaneous extraction and detection of halofuginone, diclazuril and toltrazuril sulfone from egg and meat.
- A multiplex immunoassay screening test was developed to detect 18 different aminoglycoside residues in honey samples. The developed method offers a significant improvement over other analytical methods for aminoglycoside detection and should have widespread application in other matrices including milk and meat.
- A confirmatory UHPLC-MS/MS method developed using a new Capcell-STREP column for AMGs in honey.
- A multi-class method was developed to determine antibiotics belonging to different classes including tetracyclines, sulphonamides, quinolones, and macrolides in aquaculture products.
- CIT researchers developed the first Irish tests for detecting pyrrolizidine alkaloid (PA) and their n-oxides (PANO) residues in honey, milk, cheese and herbal matrices.
- QUB developed new technologies to screen endocrine disrupting activity in sport supplements. Two validated assays, one for estrogenic compounds and one for androgenic compounds are now available.
- A new test has been developed by QUB to detect illegal use of nitromidazoles using novel marker chemistries identified in turkey and pigs.

**Residues studies**

Teagasc carried out three persistency studies on three nitroimidazole drugs (dimetridazole, ronidazole and metronidazole) in Tiger prawn samples. The studies showed that the parent drugs dimetridazole, metronidazole and ronidazole were the major residues found in prawn muscle tissue following an in-water medication. The effect of different cooking procedures (boiling, frying, grilling and microwaving) were investigated.
showing that dimetridazole was more stable than metronidazole and ronidazole. Results showed that boiling of shrimps prior to further cooking reduced nitroimidazole levels by partial extraction into the water, but did not eliminate residues. In conclusion, cooking can reduce nitroimidazole residues in food but cannot be used as a safeguard against exposure.

**National Food Residue Database (NFRD)**

A number of improvements were made to the NFRD during the project. This included an upgrade of the sequel server, development of a test instance and an automated system to upload data onto the server. During the project more than 50 datasets were uploaded onto the NFRD. In addition, four new user videos were developed to show users from different sectors how to interrogate the database. The database is the most comprehensive and authoritative source of residues in Irish food. It can be used by industry to demonstrate the safety of Irish food to international buyers. It can be also be used by Irish food processors to check potential residue problems with food ingredients that they are buying on international commodity markets.

*Veterinary Drug Residues Exposure and Risk Assessments*

Exposure assessments of the Irish population to six anti-microbials (amoxicillin, chlortetracycline, oxytetracycline, benzylpenicillin, sulfadiazine, sulfadimidine), 10 anthelmintics (abamectin, albendazole, closantel, fenbendazole, ivermectin, levamisole, moxidectin, nitroxinil, rafoxanide, triclabendazole), and five anticoccidials (decoquinate, lasalocid, monensin, narasin, nicarbazin) were complete. Worst case scenario deterministic approaches yielded no ADI exceedance in any instance and exposure levels decreased when refined, probabilistic methods were applied.

*Exposure to food additive intake*

Three food additives were identified for assessment of intakes: nitrates, nitrites and sulphur dioxide. Mean intakes were below the ADI for each of the additives.

*Sub-project 2: Nutrition and Health*

*Maintenance of the National Food Ingredient Database*

A comprehensive food ingredient database was developed and employed to produce a report on patterns of food additives in Irish foods, which was supplied to the Food Safety Authority of Ireland. Exposure assessments to artificial sweeteners were also completed. Intakes of the four sweeteners (aspartame, saccharin, acesulfame K, sucralose) were within the Acceptable Daily Intake levels for preschool children. This ingredient database is collected at a brand level with information directly relevant to industry. The database can be interrogated to assess patterns of occurrence of any food ingredient e.g. additives, nutrients. Furthermore, the details of these brands are directly linked with food consumption data where by intakes of each food and food ingredient can be assessed. Ireland is unique insofar as having such a detailed food ingredient database that is linked to food consumption data and can be used for refining exposure assessments.
Irish Supplemental Food Composition Database

The updated Irish Supplemental Food Composition Database was updated from food consumption records collected in the Irish national food consumption surveys (the National Children's Food Survey, the National Teens' Food Survey, the National Adult Nutrition Survey and the National Pre-school Nutrition Survey; www.iuna.net), which together cover food consumption from age 1 - 90 years. It was developed by collection of food packaging and from manufacturers’ information, and has been consistently updated during each survey, to reflect the most recent composition data. It contains data on macro- and micronutrients, including fatty acids, added sugars, folic acid, vitamin D, sodium, and carotenoids and other bioactive components, for Irish foods and recipes for composite dishes (both restaurant and home cooked foods), reformulated foods, nutrient supplements, fortified foods, and foods for young children. Data are included for a total of 1533 new or updated food codes. The database facilitates ongoing monitoring (with FSAI) of intakes of nutrients, bioactive constituents and botanical and herbal products in Ireland and underpins Ireland’s contribution to the EU level data collection required by EFSA.

A report on the patterns of consumption of herbal supplements in Irish adults has been completed. An electronic database of herbal supplements and their labelling, including the use of health claims, consumed by Irish adults was compiled. This showed that herbal (non-nutritional) supplements were consumed by 7% (n 108) of 18-90 year old adults in Ireland. A greater proportion of women (9%) were found to consume herbal supplements compared with men (6%). A total of 105 supplements were recorded as consumed. Garlic capsules were the most frequently reported (12%) followed by glucosamine (11%) and Echinacea (7%). A range of other herbal supplements was consumed including e.g. supplements such as barley grass, chlorella and bladderwrack tincture.

Sub-project 3 Development of the National Microbial database

A fully functional national microbial database was successfully developed which incorporates data on the major pathogens in Irish food generated by the major regulatory and research institutions in Ireland. This is the first time such a database has been developed and it is now fully operational with three years of data in the system. The database can be accessed online through secure login. The microbial database represents a major resource to the regulatory and research institutions in Ireland working in food safety. The database is a repository of valuable information that can be used to underpin quantitative risk assessments for a large number of product / pathogen combinations. Although the database access is restricted and industry do not have direct access to the data. The data can be used to generate risk assessments of direct relevance to the industry as the need requires. The database has also improved the communication of data between the various regulatory and research agencies working in food safety in Ireland.

The National Microbial Database was populated with over 100,000 records of individual test data collected over three years that was provided by DAFM and FSAI. Using data within the database, two preliminary risk assessments were completed as demonstration projects which quantified a demonstrable risk of norovirus in oysters, particularly during winter months. A separate risk assessment on Campylobacter in poultry showed that
better control of key steps in the slaughter process would reduce the presence of Campylobacter in poultry.

At DAFM, molecular and antimicrobial resistant (AMR) data was incorporated into the NMD using two methods. The first method used was to input character data (MLVA, other molecular data and AMR) directly into the NMD using three different fields (MLVA, additional subtyping and AMR). The second method involved the conversion of PFGE data into a character format. This involved the collection of 1233 Listeria monocytogenes (DSL and TFRC Moorepark) and 1121 Salmonella (CVRL and TFRC Ashtown) PFGE profiles. The degree of similarity between each profile was determined using Bionumerics to analyse all isolate profiles. A code was assigned to each profile, this code consisted of three pieces of information: [1] the enzyme used to restrict the DNA; [2] the G number - any profile with the same G number were 90% similar to each other; and [3] the P number - profiles with the same G and P number are 100% genetically similar, e.g. XbaI G1 P1. Within the reporting period DAFM was involved with different sub projects within the CVRL and DSL laboratories, relating to research on a variety of pathogenic (VTEC, L. monocytogenes, Salmonella, Campylobacter and Mycobacterium bovis) bacteria. This involved the molecular characterisation, virulence profiling and antimicrobial resistance profiling of cultures from both the Listeria monocytogenes and Salmonella National Reference Laboratories (NRLs).

The feasibility of linking Northern Ireland foodborne pathogen data into the proposed all-Ireland database was also investigated, to provide a more comprehensive overview of patterns and trends in relation to the challenges posed by these organisms. The study established the nature, content and courses of current quantitative data streams within NI, and identified the functions and activities of relevant organisations, the scales of sampling, frequencies of detection, and data destinations.

The task reviewed the NI reporting methods with regard to potential integration in an all-Ireland database. The task concluded that the systems for surveillance and reporting foodborne pathogens in NI are significantly different from the systems in RoI, which may pose challenges in relation to the inclusion of NI data within an all-island database. There were a number of practical and operational challenges identified ranging from differences between the nature and presentation formats of data in both jurisdictions, through the extent to which specific details are lost during consolidation and the preparation of summary reports, to delays related to hierarchal reporting and annual reporting cycles.

4. Impact of the Research

VetFAD was key to the production of the FSAI Scientific Report entitled "Risk-Based Approach to Developing the National Residue Sampling Plan". This report is being used by regulatory agencies to target resources more efficiently for the National Residue Control Plan.

A series of food surveys were conducted of different commodities including honey, broiler meat, eggs and farmed aquaculture. In some cases, non-compliant residues were successfully detected in samples and regulatory agents companies were informed. The analysis that was carried out on the project was as follows:
271 honey and 124 aquaculture samples were tested for nitrofurans. Non-compliant levels of semicarbazide and furazolidine (as AOZ marker) were detected in one honey sample (1.27 µg kg\(^{-1}\)) and one king prawn sample (1.6 µg kg\(^{-1}\)), respectively.

260 aquaculture, 200 egg and 183 poultry samples were tested for nitroimidazoles, all were negative.

A total of 309 honey samples and 198 poultry samples were analysed for the presence of chloramphenicol. Two honey samples tested positive, one from the UK and another from Bulgaria, both purchased at health food shops.

476 egg samples were tested for anticoccidials and two egg samples were found to be non-compliant both containing monensin at 3 µg kg\(^{-1}\) (quail egg sample from France) and 15.1 µg kg\(^{-1}\) (table egg sample from Ireland).

Coccidiostat residues were monitored in 198 broiler meat samples. One chicken breast meat sample was non-compliant containing lasalocid at a level of 54 µg kg\(^{-1}\).

Surveillance of dyes in aquaculture and poultry revealed one non-compliant sample has been found of shrimp from Thailand containing malachite Green at a level of 35 µg/kg. Traces of Crystal Violet and Brilliant Green were detected in a number of samples, but all at concentrations less than limit of quantitation.

Almost 98% of samples (289) tested negative for residues of aminoglycosides, five samples contained analytes at levels greater than the detection capability - one positive each for hygromycin B, apramycin, gentamycin, amikacin and streptomycin. Furthermore, a sample was found to be positive at a level of 832 µg kg\(^{-1}\) for streptomycin, 35 µg kg\(^{-1}\) for neomycin and 26.2 µg kg\(^{-1}\) for apramycin.

A total of 160 samples of prawn and finfish for multiple antibiotics. 137 of the samples were negative. Quinolone, tetracyclines and sulphonamides were detected in 19, 6 and 1 of the samples, respectively.

A total of 369 honey samples were tested for PAs and 24% (n = 90) were positive for one or more PAs and/or PANOs. The mean concentration of the total sum of PAs detected in positive honey samples purchased in Ireland was 81 µg kg\(^{-1}\) with a median of 26 µg kg\(^{-1}\). Honeys classified as blends of EU and Non-EU honeys had the highest frequency of positives (27%) but samples originating from Australia and New Zealand had the highest concentrations detected (1147 and 546 µg kg\(^{-1}\), respectively).

PAs and PANOs were also analysed in the herbal teas and TCMs. The results reported represent the minimum levels of PAs and PANOs present in the samples analysed: commercially available herbal teas (n=18) and herbal medicines (n=54). A total of 50% herbal teas and 78% herbal medicines tested positive for one or more PAs and/or PANOs included within this study, ranging from 10 to 1733 and from 13 to 3668 µg kg\(^{-1}\), respectively.
5. **Exploitation of the Research**

Methods developed on the project have been accredited at Teagasc and AFBI laboratories to support ongoing residue surveillance.

Ingredient and food compositional databases are being protected under IUNA and have potential to be exploited in the development of new healthier food products.

Discussions have been held with DAFM animal health and welfare division regarding the continuance of VetFAD.

The NFRD and Central Microbial Database continue to be maintained by Teagasc and UCD.

6. **Summary of Research Outputs**

(a) Intellectual Property applications/licences/patents

Not applicable.

(b) Innovations adopted by industry

1. The new VetFAD database is being used regulatory agencies and by industry to prioritise residues surveillance activities.
2. The multi-residue UHPLC-MS/MS method for anticoccidials is now widely used to monitor residues in food samples collected as part of the NRCP and self-monitoring by industry.
3. The nitrofurans UHPLC-MS/MS method is used for the monitoring of residues in honey and aquaculture samples collected as part of the NRCP.
4. The nitroimidazoles UHPLC-MS/MS method is used for the monitoring of residues in aquaculture samples collected as part of the NRCP.
5. The multi-antibiotics method is being used on the safefood food risk register project to analyse residues in pork samples collected from industry.
6. The National Food Residue Database is being used as a tool by the food industry for risk analysis purposes and to demonstrate the purity of Irish produce to international buyers.
7. INFID has been used to prepare a report on patterns of food additives in Irish foods for the Food Safety Authority of Ireland.
8. The Supplemental Food Compositional Database is being used to facilitate ongoing monitoring of intakes of nutrients, bioactive constituents and botanical and herbal products in Ireland and underpin Ireland’s contribution to the EU level data collection required by EFSA.
9. The central microbial database is being used by research and regulatory agencies to collate data during ongoing monitoring of food.

(c) Number of companies in receipt of information

A total of 55 companies have participated on workshops on this project.

(d) Outcomes with economic potential

1. Residue surveillance research on this project is important to support the trade of Irish food products on EU and International Markets.
2. The Food Ingredient and Food Compositional Databases can foster entrepreneurship through their use in the development of healthier and safer food products.

(e) Outcomes with national/policy/social/environmental potential
1. The VetFAD database can be used as a tool to collect data on the usage of veterinary drugs in different animal species on the island. It is also useful to monitor the amount of chemicals that are being transferred to the environment.
2. A risk ranking of veterinary drug residues was carried out using reports prepared from the NFRD and the VetFAD. This can be used in the future to target the most important residues to monitor in food.
3. Analysis and exposure assessments for veterinary drugs and pyrrolizidine alkaloids are valuable for risk assessment of residues in food. The new analytical methods developed on the project are being used on an ongoing basis to improve the safety of food products consumed and produced on the island.

(f) Peer-reviewed publications, International Journal/Book chapters.


(g) Scientific abstracts or articles including those presented at conferences


(h) National Report

(i) Popular non-scientific publications
2. Rae, J et al. Article in the 'Irish Exporters Association' June 2013 Newsletter.

Four user NFRD user videos were uploaded onto Youtube in 2013.
1. Meat
2. Seafood
3. Fruit and vegetables.
4. Dairy

(j) Workshops/seminars at which results were presented (excluding those in (g))
5. Safe & Healthy Foods Workshop, Teagasc AFRC, 22nd May 2012.
7. **Permanent Researchers**

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8. **Researchers Funded by FIRM**

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9. **Postgraduate Research**

Total Number of PhD theses: 4


Total Number of Masters theses: 2


10. Involvement in Food Graduate Development Programme

Not applicable.
11. **Project Expenditure**

Total expenditure of the project: €4,279,761.85

Total Award by FIRM €4,451,999.30

*Other sources of funding (specify)* €

1. 
2. 

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<td>2057892.55</td>
<td>278578.24</td>
<td>164491.04</td>
<td>4279761.85</td>
</tr>
</tbody>
</table>

Breakdown of Total Expenditure
12. Future Strategies

Outline development plans for the results of the research.

Analytical methods
- The analytical methods developed by Teagasc and AFBI have been largely validated and accredited to ISO17025 standard. These methods are available to industry and regulatory agencies for residue surveillance purposes.
- CIT have received additional funding for PA research and will continue work on this topic in collaboration with Teagasc over the next four years. Teagasc will investigate the accreditation of this in the future.

Survey results:
- Teagasc/CIT plan to prepare additional papers on the analysis of coccidiostats, aminoglycosides and PAs.
- Data from these surveys are currently maintained at Teagasc and CIT.

Databases
- Discussions have been held with DAFM Animal Health and welfare Division regarding the continued population of VetFAD.
- The NFRD will continue to be hosted by Teagasc into the foreseeable future. Additional funding will be needed to keep the NFRD current.
- The Food Ingredient and Food Compositional Databases are maintained protected under IUNA.
- The central microbial data base is currently by UCD and will require further funding to maintain it into the future.

13. Industry Collaboration

Extensive links were developed with the major egg packers on the island, which resulted in a joint industry survey of anticoccidials in eggs.

Similarly a survey of nitroimidazoles was conducted in broiler meat in collaboration with the poultry industry.

Surveys of veterinary drugs and PAs were carried out in honey. Results were communicated back to stakeholders (honey producers and health food stores) through workshops and analytical reports.