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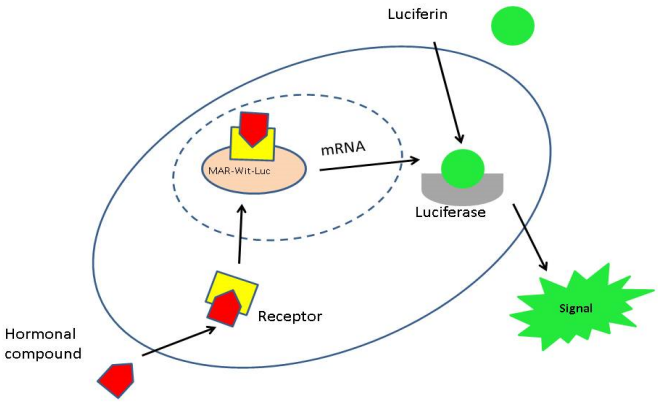
Detection of Endocrine Disrupting Agents in Milk



Reporter Gene Assay System

Key external stakeholders:

Dairy industry, Dairy farmers, Agri-businesses, Policy makers

Practical implications for stakeholders:

Endocrine disruptor agents (EDAs) comprise of both naturally occurring and synthetic chemicals. Some of these chemicals can transfer into milk due to environmental contamination, feed contamination, leaching from milking machine components, cleaning agents or processing. This research has shown that endocrine disruptors can be successfully detected in milk using receptor assays. However, chemical analysis using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is required to accurately measure and identify each compound. Unfortunately, a wider range of EDAs could not be detected because there are more amenable to GC-MS analysis, which was not available at the time.

Using the technology developed on this project low levels of EDAs were found in milk samples but further investigations should be carried out to identify the source of residues. More extensive methodology is required to properly investigate a wider range of phthalates, which have been detected in dairy products in other EU countries.

Main points

- The technology developed on the above project provides two validated solutions for detecting EDAs in milk.
- End-users can use the technology to screen for endocrine disrupting chemicals in milk and be confident that dairy is safe for consumption.

Main results:

- Two new methods were developed to analyse endocrine disrupting agents in milk using an estrogenic reporter gene assay and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).
- The technologies were applied to a range of different types of milk and infant formula.
- A range of endocrine disruptors were detected in samples including the natural hormone progesterone and low levels antimicrobials, phytoestrogens and benzyl butyl phthalate.

Opportunity / Benefit:

This technology is now available as a tool to monitor the safety of milk.

Collaborating Institutions:

Queen's University Belfast

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Teagasc project team: Dr. Martin Danaher (PI)
External collaborators: Dr Lisa Connolly, Queens University Belfast

1. Project background:

Endocrine disruptors are chemicals which mimic natural hormonal compounds or that can otherwise interfere with normal endocrinal function and have significant implications for human health, being implicated in cancers, malformations, infertility and obesity. A large number of chemicals can be classed as endocrine-disrupting. Some of these are natural substances, such as endogenous hormones and phytoestrogens; others are artificial in origin, such as pesticides, bisphenol A, phthalates, alkylphenols and persistent organic pollutants.

In the research project, two different approaches were employed to detect and measure endocrine disrupting chemicals in milk. The first was a targeted test based on liquid chromatography coupled to tandem mass spectrometry, which is suitable for accurately quantifying known endocrine disruptors. The other approach was based on cell based assay, which can detect the overall endocrine disruptor activity and is suitable for unknown agents.

2. Questions addressed by the project:

- Can methodology be developed to detect endocrine disrupting agents in milk?
- What endocrine disruptors are present in milk and at what levels?

3. The experimental studies:

This research project set out to assess biological activity of different contaminants, which gain entry to the food chain as well as analyze a variety of milk samples for their total estrogenic hormonal load and chemical composition.

The assessment of environmental contaminants was performed employing an estrogenic reporter gene assay (RGA). Milk samples were analyzed using two assays, namely, a screening reporter gene assay and a quantitative LC-MS/MS method which were validated according to 2002/657/EC guidelines.

4. Main results:

A number of contaminants were evaluated in the reporter gene assay and showed activity including UV filters, parabens, phthalates, pyrethroid pesticides and their metabolites, stressing a possible risk for consumers if exposed to mixtures of these compounds.

The development and validation of both screening and confirmatory methods yielded two fast and highly reliable assays with suitable sensitivity for screening for estrogenic activity above the levels resulting from the presence of endogenous hormones as well as chemical confirmation of nineteen EDAs in milk at sub parts per billion (ppb) levels.

Results of the screening of a range of milk samples revealed the presence of low level mixtures of EDAs of natural origin, such as myco- and phytoestrogens, but also man-made chemicals such as antimicrobials. Nevertheless, the chemical contamination did not translate to enhanced estrogenic hormonal load in majority of samples. Only 3% of those tested showed increased estrogenic load, which origins have not been confirmed.

Employment of fractionation with subsequent concomitant biological and untargeted chemical analysis was investigated as an additional tool which could provide invaluable insight into the composition and possible origin of the biological activity detected in milk. Additionally, analysis of masked EDAs present in milk have been performed by samples reassessment with the inclusion of an enzymatic deconjuation step and revealed higher concentrations of contaminants resulting in an increased estrogenic load.

The research highlights the importance of ongoing screening of food commodities for EDA contamination and highlights the advantages of employing combined biological and chemical assays to facilitate accurate risk assessment.

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5. Opportunity/Benefit:

- Two new tests have been developed on this project and can be used by the dairy industry to measure endocrine disrupting compounds in milk.
- These tests can be used for public good by the dairy industry to improve the quality and safety of milk and dairy products produced on the island.

6. Dissemination:

PhD Thesis

Wielogorska E., Developing assays to detect and quantify endocrine disrupting compounds in milk. (November 2014) PhD Thesis. Queens University Belfast.

Main publications:

Wielogorska E., Elliott, C.T., Danaher, M and Connolly, L. (2014) 'Validation and application of a reporter gene assay for the determination of estrogenic endocrine disruptor activity in milk' *Food and Chemical Toxicology* 69:260-266.

Wielogorska E., Elliott, C.T., Danaher, M., Chevalier, O and Connolly, L. (2015) 'Validation of an ultra high performance liquid chromatography - tandem mass spectrometry method for detection and quantitation of 19 endocrine disruptors in milk' *Food Control* 48:48-55.

Wielogorska E., Elliott, C.T., Danaher, M and Connolly, L. (2014) 'Endocrine disruptor activity of multiple environmental food chain contaminants' *Toxicology in Vitro* (in press).

Popular publications:

Wielogorska E., Elliott, C.T., Danaher, M., Chevalier, O and Connolly, L. 'Validation of an ultra high performance liquid chromatography - tandem mass spectrometry method for detection and quantitation of 19 endocrine disruptors in milk.' ASSET Food Integrity and Traceability Conference, 8th – 10th April 2013, Belfast, UK.

Wielogorska E., Elliott, C.T., Danaher, M., Chevalier, O. and Connolly, L. 'Endocrine disruptor activity of multiple environmental food chain contaminants' ASSET Food Integrity and Traceability Conference, 8th – 10th April 2013, Belfast, UK.

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