**Overview: An epidemiological evaluation of all relevant data gathered to date and an assessment of hypotheses that could plausibly be associated with poor animal performance on the index farm**

S.J. More[a], E.A. Lane[a,b,c], M.J. Canty[a,c,d], G. McGrath[a] and M. Sheridan[b]

[a]Centre for Veterinary Epidemiology and Risk Analysis (CVERA), Veterinary Sciences Centre, University College Dublin, Belfield, Dublin 4, Ireland

[b]Department of Agriculture, Fisheries and Food, Agriculture House, Kildare St, Dublin 2, Ireland

[c]Veterinary Inspectorate
[d]Agricultural Inspectorate

**Abstract**

Long-term problems of cattle performance were observed on a farm (the 'index farm') in Co. Kilkenny, Ireland. In recent years, a number of investigations have been conducted. The objectives of this paper are to critically evaluate all relevant data, and to assess hypotheses that could plausibly be associated with these problems of animal performance. A number of general hypotheses were developed, each potentially associated with case development, including hypotheses specific to the index farm (health, management, genetics, nutrition) and broader hypotheses (element imbalances). Each hypothesis was then assessed by considering all available sources of data, taking account of data validity and precision, and using the Bradford-Hill criteria for evidence of causation during decision-making. A number of essential element deficiencies have been identified in soil, herbage and cattle on the index farm, including calcium, copper, selenium and zinc. Observed soil conditions (low pH, high liming requirements) are known to adversely affect the availability of essential elements. The deficiency in cattle was not responsive to Se supplementation, consistent with an aetiology more complex than Se alone. A number of animal health concerns were identified on the index farm prior to 2007, with adverse effects on animal performance. Since 2007, however, the potential for such effects has been substantially reduced, following the introduction of robust preventive measures and as a consequence of intensive veterinary supervision by the project team and the farmer's own private veterinary practitioners. There is evidence of elevated background concentrations of Cd among cattle on the index farm. However, these concentrations are not of toxicological significance based on currently knowledge, and would not be expected to adversely affect animal health. Fluorine is present intermittently, but not at concentrations considered detrimental to animal health. A number of factors have been described that undoubtedly have some influence on the performance of cattle on the index farm. However, no comprehensive understanding has yet been established to fully explain the epidemiological presentation of cases, in particular the localisation of cases to the index farm and the temporal distribution of periods when negative performance was recorded.

## 1. Introduction

Long-term problems of cattle performance were observed on a farm (the ‘index farm’) in Co. Kilkenny, Ireland. These problems presented intermittently principally during the early 1990s, but were reported to have progressively worsened since 1997. There is some anecdotal evidence (from a private veterinary practitioner) that the problem may have presented as early as 1979, when small statured cows were observed. The problem principally presents as stunted growth and ill-thrift in growing cattle, although poor body condition and reduced milk yield in adult cattle were also observed. Marked stunting was generally first noted in calves after the first few weeks of life, with calves gaining as little as ~0.18 kg/day during the first year of life (More, 2005 [Appendix 6]). Animals appeared normally-proportioned, but of small stature. The problem affected both home-born and introduced young animals. Affected animals appeared to improve (in condition, but not stature) if moved away from the index farm. In addition, substantial fluctuations in average daily gain of cohorts of growing stock have been reported (for example, *Study 2*). These fluctuations are not consistent year-to-year, nor are they associated with periods of insufficient nutrition. These problems may be geographically clustered, noting that poor animal performance has been reported on at least one neighbouring farm.

The index farm is located in hilly topography on the edge of the Castlecomer plateau, approximately 1 km from Castlecomer in Co. Kilkenny. The Castlecomer area is located near the centre of the Leinster coalfield (which covers an area of approximately 250 $km^2$; Higgs and O’Connor, 2005) and has a long history of anthracite coal mining, from the 1600s to 1969. The local Deerpark mine, located approximately 3 km north of Castlecomer, was the largest opencast coalmine in Ireland, producing nearly 100,000 tons of coal annually at its peak in the 1940s (Kuentzel, 2002). In this area, the soils are generally considered unsuitable for cultivation or intensive grassland production unless properly managed, due to poor to very poor natural drainage (Conry and Ryan, 1967; Gardiner and Radford, 1980). The index farm (total area 67 ha.; in 2008: 207 cattle) is divided as three separate fragments, including two that are separated by a roadway. It was run as a dairy farm for many years, and as a mixed dairy/suckler (beef cattle) operation since the early 1990s. Stock water has consistently been sourced from a disused on-farm shale quarry, except for three years from 1999 when an on-farm well was used. A programme of grassland improvement was conducted during the 1990s. The problem of cattle performance has been investigated intensively by several organisations in recent years, including the Department of Agriculture, Fisheries and Food (DAFF), Teagasc and the Environmental Protection Agency (EPA).

It is well established that adverse health events frequently cluster, either in time and/or space, regardless of whether the cause is infectious or not. A broad range of methods can be used to investigate these events, including those drawn from clinical, laboratory-based and epidemiological disciplines. Epidemiological methods are generally well-suited to the investigation of event clustering, as they provide an understanding of the patterns of presentation, and an insight into clues that might potentially be associated with the source and/or cause of the problem. Results from epidemiological studies can prove helpful in guiding in-depth laboratory studies. These methods are well suited to investigation of rare diseases, to situations where exposure leads to a highly specific presentation and can be useful as a tool to determine the potential disease risk from a defined exposure (Elliott et al., 1995). With care, epidemiological approaches can also be used to investigate single-site clusters (so-called small area studies; Elliott et al., 1992).

In recent years, considerable data have been generated relevant to animal performance problems on the index farm, particularly in growing cattle. A broad range of methodologies have been used, both epidemiological and otherwise. The objectives of this paper are to critically evaluate all of the relevant data, and to assess hypotheses that could plausibly be associated with the problems of poor animal performance on the index farm.

## 2. Materials and methods

### *2.1 Data collection*

There is a considerable body of data relevant to the animal performance problems on the index farm. For the current review, we first identified all relevant studies, and from each distilled the key findings and conclusions.

### *2.2 Hypothesis formulation*

A number of general hypotheses were developed, each potentially associated with problems of animal performance in growing cattle, based on the opinion of national experts and after considering information in the general international literature (for example, veterinary texts including Radostits et al., 2007). These included:

- hypotheses specific to the index farm (such as management, nutrition, genetics and animal health concerns), as well as
- hypotheses not necessarily specific to the index farm, including essential element imbalances and environmental pollution.

*2.3 Hypothesis testing*

The key findings and conclusions were assembled, and weighted after considering the validity and precision of the source from which it was drawn. As required, further data (such as national soil maps) were obtained. We also gathered detailed background information about each potential hypothesis after consulting the specialist international peer-reviewed literature (e.g., case studies of similar animal cases, methods of environmental monitoring). Then, each hypothesis was critically assessed using the nine Bradford-Hill criteria of causation (strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, experimental evidence, analogy; Hill, 1965, Rothman et al., 2008) as a guide. Consistent with comments from Phillips and Goodman (2004), cause-effect decisions were not based on a set of rules; rather, these criteria were used to assist with decision-making based on epidemiologic evidence, including biological plausibility, consistency with clinical signs and other clinical features in affected animals, and with the broader epidemiological presentation (in particular, patterns in time, in space and between different animal groups). During decision-making, we were aware of, but unable to meet, the standards of proof necessary to achieve scientific certainty (Weiss, 2003a,b).

## 3. Results and discussion

*3.1 General comments*

3.1.1 An overview

A total of nine major (including the five described above) and three minor government-commissioned studies, and three other studies, were reviewed (Table 1). These range in date from 2004 to 2009, and in scope from large government-commissioned studies (for example, DAFF, 2006) to short reports of on-farm observations. An additional seven supporting analyses and reviews were undertaken, including detailed air dispersion modelling and a review of all available literature on Cd in cattle (Table 2).

Table 1. A summary of key results and conclusions from relevant studies

| Date | Investigator(s) | Study(ies) | Key results | Key conclusions |
|---|---|---|---|---|
| **a. Major, government-commissioned studies** | | | | |
| March 2004 (Kavanagh and Cody, 2004) | Teagasc | On-farm investigations (including controlled feeding trial during winter 2003/04) | Intermittent weight gain, including defined periods when ADG was very low ADG (weanlings, 0.2-0.3 kg/day; finishing cattle, 0.43), despite adequate feeding and feed intake, housing and management. Effect similar with home-grown and bought-in silage. Low milk production. Silage/soil analysis: low Cu, Se, Zn, P; high Mo, Al, Fe, S. Normal mineral concentrations in serum. | Soil mineral imbalance and the potential for mineral interactions were highlighted as areas of concern. |
| 2005 (Cody et al., 2005) | Teagasc, DAFF | Controlled on-farm feeding trial during winter 2004/05 | Intermittent weight gain observed on the index (but not the control) farm, regardless of silage source and despite normal or above normal feed intake. Reduced performance (ADG) was observed during March-April 2005 in both home-bred and introduced animals. | There was a marked location effect, unrelated to silage source, on ADG during defined periods of the controlled trial. The effect was measured in both home-bred and introduced animals. |
| June 2006 (DAFF, 2006) | DAFF | On-farm investigations, laboratory submissions (2003-2006) | No clinical, pathological or analytical evidence of fluorosis. A number of problems were identified following detailed review of laboratory, on-farm and other data, relating to the health of calves and growing stock (problems of passive transfer of | There was no evidence of fluorosis. There was an ongoing problem of infectious disease, particularly among young stock, including acute and chronic pneumonia and *Salmonella* |

| | | | | |
|---|---|---|---|---|
| | | | immunity, gastro-enteric pathogens, sub-clinical pneumonia, chronic broncho-pneumonia, potential concerns about BVD and salmonellosis) and adult cows (mastitis, nutritional deficits, liver fluke infestation). | *dublin* infection. |
| September 2006 (EPA, 2006) | EPA | Work completed by the EPA concerning the environmental impact of emissions from the brick factory | A review of factory processes and the IPPC (Integrated Pollution Prevention and Control) license. Further, a range of studies were conducted, including: (1) Examination of fluorine emissions from the factory, using stack monitoring, air dispersion modelling and ambient air monitoring; (2) Examination of fluorine in the environment using soil and grass survey, an ecological survey (EPA/UCC; see below), a tree survey and surface and groundwater monitoring; and (3) Examination of operations in the factory, including process modification, stack height, raw materials, additional monitoring of air emission and monitoring of minor emissions. | The environmental impact of emissions from the brick factory, particularly fluorine, was not sufficient to cause significant environmental pollution or animal health problems. |
| November 2006 (UCC, 2006) | EPA/UCC | Ecological assessment of epiphytic lichens (March/April | There were differences in lichen diversity and distribution at varying distance from the factory. Lower numbers were recorded in the vicinity of the factory. Within 650m, there was evidence of | The factory has had a significant adverse influence on epiphytic lichens within about 650m. There was no definite trend on lichen diversity and distribution at |

| | | | | |
|---|---|---|---|---|
| | | 2006) | fluorine pollution, based on both observation (lichen species and morphological types) and accumulation analysis (30-48 µg/g). However, the effect was less marked that has been reported around known sources of fluorine pollution (ie aluminium smelters). Indicator lichen species suggest moderate effects of $SO_2$ pollution at all sampling sites. | greater distances. |
| April and September 2007 *[Study 4]* | UCD | Soil and herbage survey | Low soil pH and high soil liming requirements were identified in the locality. Element deficiencies were identified in both soil (Cu, Se) and herbage (Ca, Cu, Se, Zn); the deficiencies in herbage were insufficient to meet animal requirements, particularly of dairy cows. Herbage F was variable, both in time and space, with several sample points exceeding the maximum tolerable level for cattle. F was most likely from anthropogenic activity. | The study identified element deficiencies (Ca, Cu, Se, Zn), which could have hindered optimal animal performance on the index farm. |
| January-May 2007 *[Study 1]* | UCD | Winter trial | Significant weight differences between home-reared and purchased cattle at trial start. Acceptable growth performance was observed in all cattle groups throughout the trial. A range of parameters were lower in index sourced versus | Selenium deficiency may have been a contributor to the ongoing performance problems on the index farm. Low serum IGF-1 in the index cattle was suggestive of a fundamental alteration in the |

| | | | | |
|---|---|---|---|---|
| | | | control cattle, including IGF-1, Se and GPx, Fe, several haematological parameters (RBC count, haemoglobin concentration, PCV). Elevated blood Cd concentration was measured towards the end of the study period. | somatotrophic axis, thus limiting their potential for growth. Cadmium exposure was noted, although its significance was unclear. |
| May 2007 to April 2008 *[Study 3]* | UCD | Field trial (young growing stock), evaluating selenium and/or vitamin E supplementation | Se supplementation led to an increase in whole blood Se and glutathione peroxidise concentrations; however, there was little impact on average daily gain. A dramatic rise in blood Cd was measured in all treatment groups (maximum 14,003 [group mean 4,875] µg/kg). Radiodense growth retardation lines were also observed in 6 of 9 calves. | The condition was not Se responsive, which suggested a more complex aetiology. Cadmium excess was reported in all treatment groups; on balance, the peak Cd values are considered invalid. There was also evidence of background Cd exposure. |
| January 2007 to May 2008 *[Study 2]* | UCD | Longitudinal study (older growing stock) | Weight loss/minimal weight gain during summer grazing in 2007, acceptable weight gain otherwise. Selenium and Cu deficiency among cattle, after some months on index farm. Increased blood Cd concentrations (maximum 1199.4 µg/kg) were observed in August 2007. | Cadmium excess was identified in cattle on the index farm; on balance, the peak Cd values are considered invalid. Deficiencies in essential elements were again identified as potential contributors to the lowered performance. |
| **b. Minor, government-commissioned studies** | | | | |
| 2004 (Crilly, 2004) | Teagasc | Element concentration | Samples collected from roof gutters and quarry pond sediment. Cadmium concentration in the roof gutter samples is in the range associated with | The findings highlighted the need for further investigation of Cd status on the index farm |

| | | | | |
|---|---|---|---|---|
| | | | moderately polluted soils, and well above levels recorded in herbage from uncontaminated sites. | |
| December 2005 (Gardiner, 2005) | Private consultant | On-farm examination of tree damage during September 2005 | Evidence of severe past damage, and subsequent recovery and regrowth, confined to Holly *(Ilex aquifolium)*, Hazel *(Corylus avellana)*, Ash *(Fraxinus excelsior)*, and possibly also Whitethorn *(Crataegus monogyna).* The damage was highly localised, but not uniform. Based on observation, the die-back does not appear to be caused by any fungal, bacterial or insect pest, but is consistent with pollution damage. Results of the foliar fluorine analyses are inconclusive ([F] higher on the index farm compared to 30 miles away; on the index farm, [F] similar on seemingly healthy and severely damaged trees) | Many trees suffered severe damage in the past. It was not possible to determine whether the damage was caused by atmospheric pollution or otherwise. |
| November 2007 (Gardiner, 2007) | Private consultant | On-farm botanical investigation (August 2007) | The results of foliar analysis indicated normal [F] (4.8-17.7 mg/kg), [Cl] and [S]. The result of soil analysis highlighted satisfactory pH, but very low [P] and low to very low [K]. The tree damage is not fully explained by the results of the foliar and soil analysis. There was no physical evidence of insects or fungi, and the spatial presentation was not consistent with bacterial or viral attack. | The analysis of foliage and soil did not provide any definitive explanation for the observed damage. One could speculate that the damage was caused by a combination of meteorological conditions, possibly combined with a deficiency of mineral nutrients. |

| | | | | |
|---|---|---|---|---|
| **c. Other studies** | | | | |
| March 2005 (More, 2005) | UCD | Epidemiological review of all work-to-date | Detailed epidemiological review of all available data (clinical presentation, nutrition, on-farm management, laboratory results, human health, the local brick factory). The problem appears highly clustered in space. By elimination, environmental factors (including the local brick factory) need to be considered in greater detail. | A range of additional studies were recommended (performance on neighbouring farms, questions relevant to the EPA, detailed post-mortems, digestibility trial, human health) |
| September 2005 (White, 2005) | Environmental scientist | On-farm observations (hedgerows) | Reduced lichen abundance on index farm in comparison with distant sites. Dieback and defoliation of hedgerow trees and shrubs in elevated fields on the index farm. The pattern is patchy and localised | Signs were consistent with periodic exposure to air-borne pollution |
| February 2009 (White, 2009) | Environmental scientist | On-farm observations (hedgerows) | During 2007-08, there was visible damage (to foliage and/or crown) to trees and shrubs in hedgerows on the index farm. The effect was localised, being most severe in a defined area at higher elevations towards the southeast of the index farm. | The spatial presentation of damage was consistent with air-borne pollution, rather than bacterial or fungal infection or insect damage. |

Abbreviations: DAFF, Department of Agriculture, Fisheries and Food; EPA, Environmental Protection Agency; UCC, University College Cork; UCD, University College Dublin

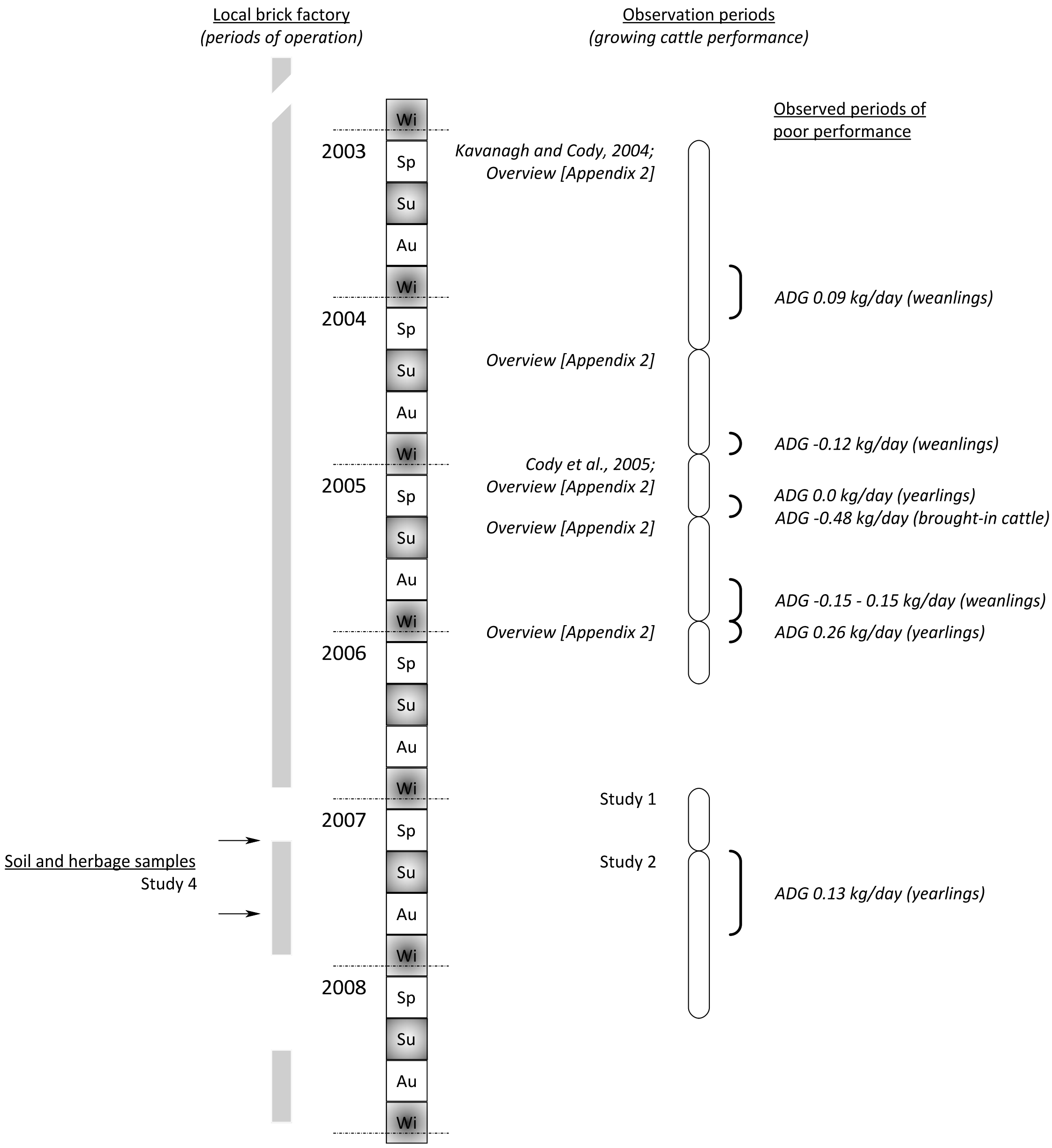
Table 2. Additional work carried out by the authors in support of the five primary studies

| Appendix | Study focus | Key results |
|---|---|---|
| 1 | Young stock performance | During 2003-2005, in weanling and yearling animals, the ADG varied between acceptable weight gain (>0.6 kg/day) to weight loss (< 0 kg/day) during different periods of observation. Poor (< 0.3 kg/day) or negative ADGs were observed during winter 2003, winter 2004 and spring 2005. |
| 2 | Milk production | In 2008, milk yield on the index farm was on average 72% of the national average and 52% of the average among milk recorded herds. Between 1994 and 2005, somatic cell counts have moved around an average of approximately 400,000 cells/ml. |
| 3 | Antibiotic usage | During 2002-2004, the usage of injectible prescription antibiotics on the index farm was similar to other farms with similar production systems. This comparison was made based on the total volume of antibiotic dispensed, and after accounting for product formulation, dosage rate per kg liveweight and mean livestock units on the farms over the three year period. |
| 4 | Animal performance on neighbouring farms (interview) | Within the locality of the index farm, most farmers reported normal livestock growth rates. Concerns were raised by only two farmers, who each highlighted shortfalls in stock performance on their farms. |
| 5 | Quantitative assessment of dairy cow performance within the Castlecomer locality | During 2005-2008, the index farm had the lowest mean predicted 305 day lactation milk yield (kgs), and during 2005-2007, the lowest mean 24 h milk yield by stage of lactation of all the farms examined. In all four years, mean SCC by stage of lactation on the index farm were below 400,000 cells/ml. There was some evidence of negative energy balance on the index farm, however, similar results were found on a number of other local farms during the period of interest. During the study period, there is evidence of milk fat depression in mid-lactation (which is suggestive of sub-acute ruminal acidosis) on several farms, with the index farm being most severely affected. |
| 6 | Air dispersion modelling | Predicted maximum annual gaseous ($SO_2$) exposure tended to be highest at three sites: a well- |

| | | |
|---|---|---|
| | | defined area immediately NNE of the factory (in forested land), and areas to the west (which included the index farm) and, to a lesser extent, the east. Predicted gaseous ($SO_2$) exposure on the index farm was consistently high. Several other farms were similarly exposed. Predicted relative exposure in the farmyard of the index farm was not among the highest of farmyards in this locality. Therefore, predicted exposure on the index farm is greater on areas of the farm other than the farmyard. There was no evidence that predicted farmyard exposure was clustered in time. |
| 7 | Cadmium exposure in cattle: a review | A detailed review of the international literature on cadmium in cattle, including dynamics of absorption, blood and tissue Cd concentrations and the interaction between Cd and essential elements. |

The clinical presentation was as described by the herdowner. Growing animals are very small in both size and stature, but well-proportioned apart from a slightly oversized head (More, 2005 [Appendix 5]). At the start of the winter trial in late 2006, for example, the mean (SEM) weight of 9-12 month old cattle from the index farm and from a farm in Co. Meath was 166 (± 5.3) and 279 (± 5.6) kg, respectively *(Study 1).* Detailed weight data were collected from weanling and yearling animals for varying periods during 2003-2008. Average daily gain (ADG) varied substantially between acceptable weight gain (>0.6 kg/day) to weight loss (< 0 kg/day) (Figure 1). Poor (< 0.3 kg/day) or negative ADGs were observed during winter 2003, winter 2004/05, during spring 2005, autumn/winter 2005/06 and during summer/autumn 2007. Animals generally appear in good health with a good appetite, apart from stunted growth, a rough hairy coat, and (in some animals) diffusely brown teeth and pale mucous membranes (More, 2005 [Appendix 5]).

Figure 1. A timeline of selected events during 2003-2008, including observation periods (growing cattle performance), observed periods of poor performance, periods of operation of the local brick factory and dates of sampling for the soil and herbage study *(Study 4).* Poor performance was considered to occur when average daily gain in young stock was less than 0.30 kg/day



Within the locality, farmers believe that the problem is localised, predominantly affecting cattle on the index farm. It has been difficult to rigorously test this hypothesis, as only limited objective data on growing cattle performance are available from other farms in this locality *(Appendix 4)*. During local farm visits in August 2006, production shortfalls in growing cattle were reported by farmers on 2 of 8 neighbouring farms. On one of these contiguous farms, cattle achieved an ADG of 0.38 kg/day during 2002 to 2005, based on an analysis of mart records. Analysis of milk recording data has provided one method to objectively assess the relative performance of animals on the index farm, albeit in adult rather than growing cattle. However, we do caution that differences in milk production may be influenced by farm differences in management or animal genetics. The milking cows on the index farm are predominantly British Friesian, with moderate genetic potential for milk production. Milk yield on the index farm was consistently lower than that recorded on other dairy farms in the locality. The index farm had the lowest mean predicted 305 day lactation milk yield (during 2005-2008) and the lowest mean 24 hour milk yield by stage of lactation (during 2005 to 2007) of 13 milk recording farms within the locality *(Appendix 5)*. Further, milk yield on the index farm from 2000 to 2005 was on average 72% of the national average and 52% of the average among milk recorded herds (Table 2, *Appendix 2*). These comparisons provide qualified support for the presence of a localised problem. Further work to characterise the spatial extent of the problem may be justified, based on prospective collection of relevant data.

### 3.1.2 Cattle production on the Castlecomer plateau

The soils of the Castlecomer plateau have been reviewed in *Study 4*. They are predominantly gleys, with poor to very poor internal drainage, which are generally unsuitable for cultivation or intensive grassland production. A range of factors work to curtail both the grazing season and the proportion of fodder utilised, including retarded grass growth in spring and general susceptibility to poaching damage by grazing stock. Nonetheless, high levels of pasture production can be achieved on some farms in the locality, with attention to fertiliser and grazing management.

*3.2 Hypotheses specific to the index farm*

3.2.1 Animal health

A detailed investigation of defined animal health conditions has been conducted on several occasions. For some years prior to 2007, a number of infectious disease problems were identified by the Regional Veterinary Laboratory (RVL), particularly among young stock, including acute and chronic pneumonia and infection with *Salmonella dublin* (DAFF, 2006). During 1998 to 2001, the estimated mortality rate among young calves was 40%. These authors raised concerns about the incidence of clinical disease (based on laboratory submissions and antibiotic usage) and of some aspects of calf management (in particular, mixing of age groups). In the latter part of 2005, a range of improved disease management and biosecurity procedures were put in place on the index farm, including milk recording, implementation of a comprehensive herd health programme, provision of calf hutches, and general advice and literature on preventive veterinary medicine (DAFF, 2006). Since 2007, there has been an observed improvement in the health of animals on the index farm, and few animal health concerns were observed, either in growing stock or adult cattle, during intensive experimental and observational work (*Studies 1 to 5*) conducted from 2007 to 2009. In *Study 3,* clinical pneumonia was reported in one case animal and chronic pneumonia in another animal during an elective post-mortem.

Animal health conditions have the potential to adversely impact on animal performance (for example, Moriarty et al., 2007), and are likely to have contributed to stunting and illthrift in some animals on the index farm. Severe bronchopneumonia was confirmed at post mortem in one such *Study 3* animal. On balance, however, animal health concerns do not, on their own, provide an adequate explanation for the observed poor performance on the index farm during the last three years. There are several reasons in support of this view. Episodes of poor performance have continued since 2006, regardless of changes in disease management and biosecurity procedures, and despite the observed reduction in clinical morbidity. The cattle mortality rate was also much lower in 2008 and 2009, compared with 2005 and 2007 (Table 3). In addition, and at odds with what might be expected with a single infectious disease or parasitic condition, the presenting problem has occurred intermittently during 2003 to 2008, without regard to either season or defined management events such as housing or grazing (see Figure 1). Periods of poor performance have also been interspersed between periods where animal performance has been entirely satisfactory. To illustrate, in *Study 2,* a period of poor animal performance (ADG 0.13 kg/day, June to October 2007, but without any observed animal health concerns) was preceded and followed by periods of satisfactory animal performance during housing (0.96 and 0.77 kg/day, respectively). The temporal presentation of these effects has also been remarkably uniform within observed animal groups, with little within-group variation in ADG at each point of measurement. Poor animal performance has also coincided with periods when preventive animal health measures on the index farm have been most-robust (for example, housing in winter 2004/05 with detailed vaccine cover, Cody et al., 2005; grazing in summer 2007 on pasture with good grass cover and with a series of anthelmintic treatments, *Study 2).* It should also be noted that general aspects of farm management, including preventive animal health, are not dissimilar to those of other farms in Ireland. . In *Study 3,* the results of a detailed post mortem of a single representative study animal were unexceptional. Essential element deficiencies (calcium (Ca), copper (Cu), selenium (Se), zinc (Zn) and, to a lesser extent, marginal iodine (I)) have been also identified in soil, pasture and/or cattle on the index farm, sufficient to adversely affect both growth and production in cattle *(Studies 1* to *4;* also see below).

Table 3. The annual crude herd mortality rate on the index farm during 2003-2008, in total and among animals ≤1 year and animals ≥7 days but ≤1 year, based on data recorded in the Animal Identification and Movement System [AIMS] of the Department of Agriculture, Fisheries and Food

| Year | Annual mortality rate (%) | | |
|---|---|---|---|
| | Total[a] | Animals ≤1 year of age[b] | Animals ≥7 days but ≤1 year of age[c] |
| 2003[d] | 4.2 | 7.0 | 4.7 |
| 2004 | 4.5 | 10.1 | 5.1 |
| 2005[e] | 9.1 | 20.9 | 19.4 |
| 2006 | 2.8 | 5.8 | 1.4 |
| 2007[f] | 6.6 (5.0) | 14.3 (9.5) | 14.3 (7.9) |
| 2008 | 3.9 | 8.8 | 5.9 |
| 2009[g] | 2.5 | 3.7 | 2.4 |

a. Total number of cattle deaths/total number of cattle present on 01JUN each year

b. Total number of deaths among cattle ≤1 year of age/total number of cattle ≤1 year of age present on 01JUN each year

c. Total number of deaths among cattle ≥7 days but ≤1 year of age/total number of cattle ≤1 year of age present on 01JUN each year

d. Includes knackery and laboratory submissions (all years) and on-farm burials (2003 only)

e. In 2005, 6 cattle from the index farm herd were submitted to Kilkenny Regional Veterinary Laboratory for elective post mortem (DAFF, 2006) and were excluded from the analysis.

f. During 2007 crude mortality rates are presented with (and without) inclusion of the three animals that are known to have died as a result of non infectious causes:

- A control animal from *Study 1* suffered a physical accident on the control farm after the end of the study
- A control animal from *Study 1* suffered foreign body pericarditis (wire) after the end of the study
- A *Study 3* animal suffered a physical accident after falling into a drain

g. Total number of cattle deaths to 31OCT09

### 3.2.2 Other hypotheses specific to the index farm

A range of additional hypotheses specific to the index farm were assessed. Based on the evidence available, none can be plausibly considered the primary cause of the observed problems of poor animal performance on the index farm:

- *Management:* No aspects of on-farm management have been identified to explain the problems experienced (Kavanagh and Cody, 2004; Cody et al., 2005; More, 2005 [Appendices 4, 5]).
- *Genetics:* There is no evidence of a genetic basis, noting that both home-bred and purchased animals, and animals of differing breeds, have been affected.
- *Nutrition:* Based on expert opinion, the poor performance of post-weaned calves and adult cattle is not related to inadequate intake of energy and protein. As one example from February 2005, during the first 12 months of life post-weaned calves achieved an ADG of ~0.18kg/day whilst adequately consuming a diet sufficient to achieve an ADG of 0.6 kg/day (More, 2005 [Appendix 6]; DAFF, 2006). Similarly, cattle achieved an ADG of 0.13 kg/day over a 5½ month period of summer grazing in 2007 *(Study 2)*; at similar times, excellent ADGs were reported in other parts of Ireland (Keane and Drennan, 2008). As illustrated in Figure 1, the temporal pattern of poor performance is not seasonally consistent (for example, during the winters of 2003 and 2004/05, but the summer/autumn of 2007), which is at odds with a nutritional problem specifically associated with either summer grazing or winter housing. During an analysis of milk recording data from 2005 to 2008, there was some evidence of negative energy balance in early lactation and milk fat depression in mid-lactation in adult dairy cattle on both the index farm and other farms in the locality. Milk fat depression in mid-lactation has been linked to sub-acute ruminal acidosis (SARA). It may be of importance on the index farm, noting that cattle on this farm were more severely affected than elsewhere *(Appendix 5).* However, the relationship between SARA and milk fat depression is both complex and inconsistent (Oetzel, 2007); therefore, these data need to be interpreted with care. Nutritional causes of milk fat depression should only be considered after account is first taken of breed, season and days in milk, and include excessive intake of saturated fats (for example, wet distillers grain), monsensin feeding and SARA. Ruminal acidosis causes milk fat depression by inhibiting bacteria responsible for fatty acid biohydrogenation in the rumen (Oetzel, 2007).

### *3.3 Hypotheses not necessarily specific to the index farm*

#### 3.3.1 Deficiencies in essential elements of animals

Deficiencies in several essential elements appear to be contributing to, but on their own do not provide an adequate explanation for, poor animal performance on the index farm. A range of element deficiencies in both soil (Cu, Se) and herbage (Ca, Cu, Se, Zn) have been identified, as well as soil conditions (low pH, high liming requirements) known to adversely affect the availability of essential elements *(Study 4).* These conditions are not dissimilar to those found on farms in other mineral-deficient areas of Ireland. These deficiencies were present throughout the survey area (the index farm and neighbouring areas), and several elements in soil and herbage (Ca, Cu, Se, Zn) were at concentrations insufficient to meet animal requirements, particularly of dairy cows (National Research Council, 2001). Se deficient soils have previously been described in a number of areas of Ireland, including counties Carlow, Cork, Galway (east), Tipperary, Waterford and Wexford (Fleming, 1978), as well as the area under study (McGrath and McCormack, 1999). Further, cattle on the index farm were Se deficient, based on *ad hoc* monitoring (DAFF, 2006) and during formal longitudinal studies on growing animals *(Studies 1 to 3).* In the latter studies, there was a progressive and profound decrease in both Se and glutathione peroxidase (GPx) concentrations when growing cattle were introduced onto the index farm. Selenium status improved when animals were moved off-farm, but decreased once these animals returned to the index farm *(Study 3).* Further, the Se and GPx status of index farm animals following barium selenate supplementation was similar to that of unsupplemented off-farm controls *(Study 3).* Animals on the index farm were also shown to be marginal *(Study 1)* or deficient *(Studies 2 and 3)* in Cu, and marginally deficient in plasma inorganic I *(Studies 1 and 3).*

In recent years, cattle on the index farm have been supplemented with a range of essential elements, including Cu, I and Se/vitamin E (More, 2005 [Appendix 4]; according to the herd owner also cobalt (Co) and Zn) without any apparent improvement in performance. An increase in whole blood Se and GPx was observed following Se supplementation, but without any concurrent positive impact on average daily gain *(Study 3).* The lack of improved performance in animals on the index farm in the presence of Se supplementation is consistent with an aetiology more complex than Se deficiency alone.

### 3.3.2 Other element imbalances

#### a. Cadmium

Cadmium excess has been measured in cattle on the index farm, from several tissues (kidney, whole blood) and over several time periods (2003 to 2005, 2007, 2009). Two patterns of presentation are presented: background Cd exposure (in each of *Studies 1, 3 and 5*) and acute Cd excess (observed during August 2007 in *Study 2*, and in July and August 2007 in *Study 3*). Each will be considered in turn.

*Background Cd exposure*

There is evidence of *background Cd exposure* among cattle on the index farm, based on data collected during periods of intensive monitoring (*Studies 1 and 3*) and as a result of ongoing surveillance of kidney samples collected from cattle on the index farm cattle (DAFF, 2006; *Study 5)*. In the former, background concentrations of Cd were observed, in both young and older growing stock *(Studies 3 and 1,* respectively). The percentage of cattle with blood Cd exceeding 5 µg/kg increased from 5% in January 2007 to 13% in March 2007 and to 22% in April 2007 *(Study 1).* In addition, at the start of the study described in *Study 3,* 28.6% of calves had blood Cd exceeding 5 µg/kg. Elevated Cd concentrations were also measured in bovine kidneys collected during 2003 to 2005 (mean 0.9 mg/kg wet weight, range 0.1 to 4.9; DAFF, 2006) and during 2009 (mean 2.3 mg/kg wet weight, range 0.5 to 3.9 *(Study 5*). The kidney analyses were conducted at the Agri-Food and Biosciences Institute (AFBI, Newforge Lane, Belfast BT9 5PX, Northern Ireland; www.afbini.gov.uk).

Based on current knowledge, there is no evidence that these kidney Cd concentrations are of toxicological significance, and therefore would not be expected to affect animal health. Certainly, there is no evidence that exposure at this level could contribute to the stunting and poor growth rates that were observed on the index farm. Nonetheless, these concentrations are much higher than those reported in a very recent pan-European study (European Food Safety Authority, 2009), with 43.5% of all sampled bovine kidneys from the index farm exceeding the maximum Cd concentrations allowable for human consumption *(Study 5)* (European Commission, 2008). No comparative data (kidney Cd) are currently available in Ireland.

*Acute Cd excess*

Sharp increases in whole blood Cd concentrations *(an acute Cd excess)* were identified during July and August 2007 in two concurrent studies, in animals less than 12 months of age *(Study 3)* and in older growing stock *(Study 2*).

The interpretation of these Cd results has proved problematic. Concern has been raised about the validity these results, principally on the grounds of biological plausibility, and sample contamination has been suggested as a possible explanation for these findings. In the following two paragraphs, we critically evaluate the evidence in support of, and refuting, acute Cd excess in cattle on the index farm.

There are a number of arguments in support of the validity of the Cd results. We have no evidence of sample contamination during sample collection. New blood collection equipment (vacutainers, needles) were used throughout the study, however, the vacutainers had not specifically been acid-washed. Contamination, if present, would reasonably be associated with either a specific person or batch of equipment. Although increased Cd was simultaneously measured in all four supplementation trial groups *(Study 3)*, the people conducting, and equipment used during, blood collection at this time were different for the groups located on the index farm (3 groups) and in Co. Meath (1 group). The methodology for sample preparation and analysis has been described previously *(Study 2).* Initial sample preparation (whole blood digestion for mineral analysis) was conducted at Independent Analytical Supplies Ltd (IAS, Bagenalstown, Co. Carlow, Ireland), in a laboratory with no known Cd source, then forwarded to INDIKATOR GmbH (D-42329 Wuppertal, Germany; www.indikator-labor.de) for elemental and heavy metal analyses, including Cd. INDIKATOR GmbH specialises in heavy metal analysis and operates to international standard ISO/IEC 17025:2005, which specifies the general requirements for the competence of laboratories to carry out tests and/or calibrations, including sampling. Initial preparation was conducted at IAS shortly after sample submission (that is, each bleed was processed separately). Subsequent sample analyses, including those when the Cd peak was measured, were undertaken at INDIKATOR GmbH over several dates. This laboratory re-ran a proportion of samples immediately after measuring the Cd peak, with equivalent findings. At the Cd peak, there was considerable variability in measured Cd between animals *(Studies 2 and 3)* evident by the large error bars, which may be inconsistent with sample contamination. A number of samples, from other sources, were analysed for Cd at INDIKATOR GmbH at the same time, however, none had similar Cd concentrations. A similar Cd peak was observed during re-analysis (to which INDIKATOR GmbH had been blinded), based on sequential testing by animal number rather than bleeding date, however assays were rerun on samples previously digested (in IAS, Bagenalstown, Co. Carlow, Ireland) and therefore the possibility of contamination during sample digestion cannot be ruled out. We have no reason to question other results from INDIKATOR GmbH results; indeed, there was a high level of agreement between Se (analyses conducted by INDIKATOR) and GPx (UCD) results, and between whole blood (INDIKATOR) and serum (UCD) Cu results *(Study 2).* If contamination of samples did occur, on balance it is more likely to have arisen during the initial stages of sample preparation. Elevated Cd concentrations had previously been noted in samples collected from material in roof gutters at the index farm (Crilly, 2004). These results must be interpreted with caution, noting that heavy metals can be an impurity in collected tank rainwater (Magyar et al., 2008). There is very limited literature about Cd toxicokinetics in cattle (a review has been prepared in Appendix 7); most reports relate to testing of healthy young cattle on a single occasion at the time of slaughter (for example, López Alonso et al., 2000; Patra et al., 2007). This is in contrast to several studies on the index farm *(Study 2 and 3)*, where poorly-performing animals have been repeatedly sampled over a significant period of time. For this reason, extrapolation from the published literature, particularly with respect to blood Cd, may be problematic. In the older growing stock, the Cd peak was coincident with a period of weight loss/poor weight gain (-0.05 kg/day during the 3 months following turnout onto summer pasture in May 2007; an average 0.13 ± 0.03 kg/day gain during the full summer grazing period [May to October 2007]) *(Study 2).* The observed performance downturn was in contrast to that documented elsewhere in Ireland for similar grazing systems (Keane and Drennan, 2008). Finally, documented cases of Cd toxicity present with a broad range of clinical signs not dissimilar to those observed on the index farm, including illthrift, periods of extreme weight loss, a rough hairy coat, dry faeces and bone changes (Powell et al., 1964).

Nonetheless, the validity of the Cd results can be questioned, for a number of reasons. As mentioned previously, the possibility of sample contamination during acid digestion cannot be ruled out. The blood Cd concentrations observed in mid 2007 (to a maximum of 14,003 [group mean 4,875] and 1,199 µg/kg among the younger and older growing stock, respectively; *Studies 3 and 2)* are higher that previously published Cd results, from cattle at industrialised sites in Spain (López Alonso et al., 2000) and India (Patra et al., 2007; Swarup et al., 2007). They are also higher than those reported following experimental oral (Lynch et al., 1976; Houpert et al., 1997; Foihirun et al., 2006) but not intravenous (Houpert et al.,

1995) administration of Cd to ruminants. It is only in studies examining the effect of parental administration of Cd in sheep (Houpert *et al.,* 1995, 1997) that whole blood Cd concentrations as high as those reported here have been observed. The association between the Cd peak and animal performance was not consistent. In older growing stock *(Study 2)* the Cd peak was coincident with a period of poor performance, whereas in younger animals (*Study 3*) the Cd peak was coincident with a period of weight gain. In one experiment *(Study 3),* the Cd increase occurred simultaneously in each of the four groups of younger stock, which would appear consistent with a common Cd source. However, one of these groups had moved from the index farm 6 weeks prior to this peak, with no Cd measurable on the 2 intervening occasions when samples were collected. There was a decrease in blood Cd concentrations from the observed peak in both older *(Study 2)* and younger *(Study 3)* cattle over a period of a few weeks, which is in agreement with an observed half life of Cd in human blood of 3 to 4 months (short component) and 10 years (long component; Järup and Åkesson, 2009). Conversely, in sheep, intravenously injected with Cd (thus bypassing the influence of the digestive tract on Cd toxicokinetics) Cd was rapidly cleared (within hours) from blood (Houpert et al., 1995, 1997). Intraveneous injection of Cd, allows the digestive tract to be bi-passed and presumably more rapid clearance from blood to the liver and kidneys. Following substantial exposure, Cd is accumulated and subsequently eliminated, mainly from the kidneys; the estimated half life in ruminants (100 to 150 days following intravenous administration; Houpert et al., 1995) is substantially shorter than in humans (10 to 30 years; Järup and Åkesson, 2009). Unfortunately, no assessment of kidney Cd in the above-mentioned animals was possible except in two animals from a control group that were submitted for an elective post mortem in November 2007. Kidney Cd concentrations in these calves, aged 8 months, were 0.12 and 0.11 mg/kg, similar to concentrations in calves reported elsewhere (Miranda *et al.,* 2001).

In conclusion, and on the balance of all available evidence (Table 4), we believe the measured Cd peaks in *Studies 2 and 3* are biologically implausible, and therefore are most likely to be invalid.

*Potential Cd sources (relevant to background Cd exposure)*

Relevant to evidence of background Cd exposure on the index farm, there are a number of potential Cd sources, both natural and anthropogenic. Each needs to be considered in relation to the measured background Cd exposure on the index farm. Natural sources of Cd include rock weathering, volcanic activity and forest fires. In Ireland, 15% of soils exceed the EU threshold limit for Cd in soil of 1 mg/kg, predominantly due to naturally high background concentrations in association with limestone shales or volcanic material (Fay et al., 2007). However, in both April and September 2007, soil Cd was less than <0.1 mg/kg (the detectable limit of the assay) in all samples collected in the proximity of the index farm *(Study 4)*. In this locality, the vulnerability of groundwater is classified as extreme (More, 2005 [Appendix 1]). Therefore, if groundwater were contaminated in association with local mine sites or otherwise, it would most likely present over a broad geographical area (leading to problems over a broader area than that seen here). Water for stock on the index farm is currently drawn from a disused shale quarry on the farm. For all years apart from 1999 to 2001, this has been the sole water source for cattle on the farm. It would appear unlikely that this single water source were associated with the problem, given the intermittent nature of the problem. Further, Cd results from farm water have been unremarkable *(Study 1)*. There are a broad range of industrial sources of Cd pollution, including metal production (drying of Zn concentrates and roasting, smelting and refining of ores), waste incineration, fossil fuel consumption and the production of rechargeable NiCd batteries (World Bank Group, 1999). Cadmium is also listed as a hazardous air pollutant (HAP) in association with brick manufacture (US EPA, 2003). In the US, it is estimated that hydrogen fluoride (HF) and hydrogen chloride (HCl) account for 2,290 tons per year (99.6 percent) of total HAP emissions, whereas the associated metals (antimony [Sb], As, beryllium [Be], Cd, Cr, Co, Hg, manganese [Mn], Ni, Pb, and Se) account for approximately 6 tons per year nationwide (0.4 percent). In each of the above-mentioned processes, Cd is transferred to the soil from the ambient air by wet and dry deposition, where it is absorbed by plants. Acidification increases the availability of Cd in soil (World Bank Group, 1999). The index farm is located near the centre of the Leinster coalfield. However, to our knowledge, coal mining had never been conducted on or immediately adjacent to the farm. Cadmium is also a recognised pollutant in phosphate fertilisers and sewerage sludges; the latter cannot be spread on soils with Cd values in excess of 1 mg/kg (European Commission, 1986). However, sewerage sludge (from off-farm sources) has never been applied on the index farm, and phosphate fertilisers have not been applied for some years. Silage Cd was within normal limits *(Study 1)*.

*Future actions (relevant to background Cd exposure)*

Routine testing for Cd in cattle offal is not currently conducted in Ireland *(Study 5),* and additional testing may be warranted, relevant to background Cd exposure on the index farm, both:

- to clarify the importance of background Cd exposure in the locality (and further afield), and
- to identify a Cd source, if present.

Kidney Cd from cattle at slaughter is likely to offer the best opportunity to assess cumulative Cd exposure in the locality (Neathery and Miller, 1975; Järup and Åkesson, 2009).

Table 4. A summary of evidence in support of, and refuting, the validity of the Cd peak that was measured during *Studies 2 and 3* on the index farm

| Evidence in support of the validity of the observed Cd peak | Evidence refuting the validity of the observed Cd peak |
|---|---|
| The Cd peak was coincident with measured period of poor performance (in one of the two cattle groups where the Cd peak was observed, the peak was coincident with a period of weight loss/poor weight gain) | Bi-location (the peak was observed simultaneously in *Study 3* cattle held at two locations [the index farm and a farm in Co. Meath]). The Cd peak was not coincident with any period of weight loss in these cattle. |
| Similar clinical signs (clinical signs of Cd toxicity in cattle are not dissimilar to those observed in cattle on the index farm) | Excessive levels observed (measured [Cd] was substantially higher than any published reports of cattle near industrial sites or following experimental oral administration) |
| No evidence of sample contamination at collection (there is no evidence of sample contamination during blood sample collection) | Inconsistent kidney data (measured kidney [Cd] in several of these animals at subsequent *post mortem* was not high) |
| Laboratory accreditation [analysis] (the Cd analyses were conducted in a specialist laboratory operating to international standard ISO/IEC 17025:2005) | Laboratory non-accreditation [extraction] (the acid digestion of blood samples for Cd analysis was not conducted in an accredited laboratory) |
| | Non-specialised collection tubes (the samples were collected in general lithium heparin vacutainer tubes, not tubes specific for essential element analysis) |

Toxicokinesis of Cd in ruminants is not well researched. A critical review of literature on this issue has been prepared as part of this report.

b. Fluorine

The F results from the current and previously reported studies are in broad agreement. There is evidence of F within this locality, albeit with considerable temporal and spatial variation and at concentrations unlikely to substantially affect animal health.

In broad terms, F emissions are associated with a range of industrial processes, including coal-firing and the production of fertiliser, aluminium, steel, bricks and glass (Cronin et al., 2000; Loganathan et al., 2003), and volcanic activity (Araya et al., 1990). The subsequent dispersion of industrial emissions is dependent on a range of factors, including the direction, velocity and frequency of prevailing winds. Long-term application of phosphate fertilisers are a widespread source of F pollution in agricultural soils (McLaughlin et al., 1996). The uptake of F by plants is generally unrelated to total soil F, except in situations where soil pH is very low (Cronin et al., 2000).

In the locality, a lichen survey was conducted during 2006 to assess environmental stress due to atmospheric pollution (Asta et al., 2002; Weinstein and Davison, 2003), in line with current EU (for example, Jeran et al., 2007) and Irish (Brodeková et al., 2006) methodologies. Changes were observed in overall diversity and abundance of epiphytic lichen, in the distribution of F- and $SO_2$-sensitive species, and during fluorine accumulation analysis (UCC, 2006). Nonetheless, the authors considered the impact to be relatively minor, noting that this area was richer in lichens than other areas known to be affected by F, such as in the vicinity of aluminium smelters (Real et al., 2003). In a detailed study of the environmental impact of emissions from the local brick factory, the EPA (2006) concluded that factory emissions, in particular F, were not sufficient to cause either significant environmental pollution or animal health problems. In the 2007 soil/herbage study, herbage F concentrations were variable, both in time and space, with only four samples (near but not on the index farm) exceeding the maximum tolerable level for cattle (40 mg/kg) in April but not September 2007 *(Study 4).* A similar pattern of herbage F in 2003 and 2004 was reported by DAFF (2006). Soluble forms of F are rapidly and nearly completely absorbed by cattle (National Research Council, 2001). Investigations have been conducted on the potential for F toxicity in cattle (DAFF, 2006; *Study 4)*, cognisant of the substantial body of relevant literature on fluorosis in cattle (for example, Hillman et al., 1979; Krook and Maylin, 1979; Suttie, 1980; Bunce, 1985; Maylin et al., 1987; Araya et al., 1990; Jubb et al., 1993). There has been no evidence suggestive of fluorosis on the index farm, based on clinical signs, pathology, radiology and F analysis (of bone and teeth) (DAFF, 2006; *Study 3)*. Dental abnormalities (including staining and pitting) were observed in cattle on the index farm, but with no greater severity or frequency than those seen in animals at a local slaughter plant (DAFF, 2006).

c. Other elements

Several element excesses were measured in herbage (potassium (K), iron (Fe), sulphur (S)) *(Study 4)*, but are likely to be of limited direct significance.

There was no evidence of other elemental excesses associated with possible toxicoses such as arsenic (As), chromium (Cr), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), thallium (Tl) or Zn in association with the index farm (DAFF, 2006; *Studies 1, 2* and *4)*.

3.3.3 The local brick factory

Established in the late 1960s and located to the northwest of the index farm, the Castlecomer brick factory manufactures a range of fire-clay bricks. Information about brick manufacture is available elsewhere, both generally (US EPA, 1997) and specific to this factory (EPA, 2006). Briefly, upper carboniferous shale and fireclay are obtained from local quarries (including the so-called '28 acre' site which had previously been used for opencast coal mining). This material is milled to a fine consistency, then extruded and wire-cut to shape. The bricks are dried in chamber dryers and fired at about 1,000°C in tunnel kilns using natural gas or oil, at different time periods. Emissions are released from a single chimney stack 24.4 m high. The factory operates under an EPA-granted Integrated Pollution Control licence, which sets emission limit values for $SO_2$, particulates, oxides of nitrogen, fluorines and chlorides (EPA, 2006).

During 2003-2008, the local brick factory was operating during most of the periods when growing cattle were being intensively monitored (Figure 1). Therefore, normal factory operations were coincident with periods of both good and poor animal performance on the index farm. In recent years, the factory has closed on three occasions (Dec 06 to Mar 07; Dec 07 to Jul 08; from Dec 08).

Air dispersion modelling of gaseous factory emissions has been conducted *(Appendix 6),* using BREEZE software (AERMOD v7.0.58, AERMET v6.2.0 and SD Analyst v2.0.56 Pro

Plus; Trinity Consultants, Dallas, TX, USA), based on emission data (EPA), meteorological data (synoptic data, Kilkenny; upper air data, Valentia; Met Éireann, Dublin, Ireland) and LiDAR terrain data (Ordnance Survey Ireland, Dublin, Ireland). This methodology has been used previously in this case (EPA, 2006), to identify potential hotspots. BREEZE AERMOD is very sensitive to the effect of terrain on air dispersion, producing a worst-case scenario in areas with steep slopes (Sidle et al., 2004). Detailed results are reported in *Appendix 6*, including:

- The spatial distribution of *predicted maximum annual $SO_2$ exposure* (the predicted maximum hourly/24 hourly/monthly high value of µg/m$^3$ $SO_2$ during each calendar year) over a 10 km grid (the study area) during 2000 to 2007,
- The spatial distribution of *predicted continuous annual $SO_2$ exposure* (the average predicted hourly concentration of $SO_2$ during each calendar year) over the study area during 2000 to 2007,
- The *predicted relative annual farm $SO_2$ exposure*, for all farms completely contained within the study area during 2000 to 2007. Farm comparisons were made based on total and average predicted exposure,
- The *predicted relative annual farmyard $SO_2$ exposure* during 2000 to 2007, for the 36 farms with the highest predicted continuous exposure score. Farmyard comparisons were made based on both predicted maximum (hourly/24 hourly/monthly high) and continuous (hourly average) exposure,
- The temporal distribution of *predicted maximum farmyard $SO_2$ exposure* (the predicted maximum hourly high value of µg/m$^3$ $SO_2$ during each calendar year) at the farmyard on the index farm during 2000 to 2007, and
- The temporal distribution of *predicted cumulative farmyard $SO_2$ exposure* (predicted hourly concentrations based on a rolling time interval of 1, 3, 12, 24h, 3, 7, 14, 21 and 28 days) at the farmyard on the index farm during 2003 to 2007.

In this work, $SO_2$ was used as a crude proxy for gases emitted from this facility.

Throughout 2000 to 2007, predicted maximum annual gaseous ($SO_2$) exposure tended to be highest at three sites: a well-defined area immediately NNE of the factory (in forested land), and areas to the east (which included the index farm, SE of the factory) and, to a lesser extent, the west. Predicted gaseous ($SO_2$) exposure on the index farm was consistently high. Several other farms were similarly exposed. Predicted relative exposure in the farmyard of the index farm was not among the highest of farmyards in this locality. Therefore, predicted exposure on the index farm is greater on areas of the farm other than the farmyard. There was no evidence that predicted farmyard exposure was clustered in time.

The index farm consistently experiences some of the highest predicted gaseous ($SO_2$) exposure of any farm in the immediate locality, with all its land in the direct environs of this factory. Therefore, the index farm would appear to be at higher risk than most other farms in the locality, in the event of a gaseous toxic emission from this facility. However, we are not aware of any direct evidence of toxic emissions from this facility, sufficient to endanger animal health. Further, several neighbouring farms, with no reported evidence of poor animal performance, experience similar levels of annual gaseous ($SO_2$) exposure. Therefore, based on the modelling results and all other relevant data, there is no current evidence, either direct or indirect, to associate the factory with the specific problems that have been investigated on the index farm. The factory was closed in December 2008, and has not operated subsequently.

### *3.4 Other issues*

In recent years, a number of small area studies relating to animal health and performance have been conducted in Ireland. An investigation was conducted into the presence and influence of Pb in the Silvermines area of Co. Tipperary (EPA, 2004; McGrath et al., 2004). Further investigations of animal health problems have been conducted at Carrick-on-Suir, Co. Tipperary (Hanrahan vs Merck Sharpe & Dohme, Irish Law Report Monthly 629, Supreme Court, 1988) and at Askeaton, Co. Limerick (EPA, 2001). Detailed animal health monitoring has been conducted in the vicinity of an industrial chemical complex in Cork harbour for some years (Berry et al., 2005; Buckley et al., 2007). A nationally-agreed protocol for the investigative approach to serious animal/human health problems is described (EPA, 1997). Environmental epidemiological investigations relating to animal health and performance are reported from a number of countries, including Canada (the sour natural gas industry, natural gas containing significant amounts of $H_2S$; Waldner, 2001; Waldner et al., 2001; Scott et al., 2003a,b) and the Republic of South Africa (the vanadium mining industry; Gummow et al., 2006a,b).

There have been considerable advances in methods to investigate small area studies (for example, Elliott et al., 1992). Nonetheless, study results must be interpreted with caution, and confirmatory evidence is generally required before a causal association can be accepted. In veterinary medicine, a number of small area studies have now been documented. Waldner

(2001) used case studies (that is, a before-and-after comparison but with no contemporaneous control) to investigate the impact of the sour gas industry on cattle health and productivity. The effect(s) of exposure has been monitored both directly, using biomarkers [eg total sulphation, $H_2S$] (Waldner et al., 2001; Gummow et al., 2006a,b), and indirectly through effects on livestock health and productivity (Waldner, 2001; Scott et al., 2003a,b). In studies to assess the impact of the vanadium mining industry on animal health, Gummow et al. (2006a,b) observed direct effects of exposure on sentinel animals. It is important to note that formal observational methods, such as those used by Scott et al. (2003a, b) to investigate the effect of sour natural gas industry over a broad area of Canada, are generally not suited to small area studies.

This report presents data from a broad range of sources, from anecdotal information through to controlled field trials. As described previously, each hypothesis (animal health concerns, other hypotheses specific to the index farm, etc) was assessed using a clearly defined process: data from all available sources were considered, the credibility of each data source was assessed based on its validity (internal and external) and precision, the Bradford-Hill criteria were used to guide hypothesis assessment (Hill, 1965; Rothman et al., 2008), and 'on the balance of probability' or 'on the preponderance of the evidence was used as the standard of proof during decision-making (Weiss, 2003a,b). Qualitative and quantitative data were collected, where possible, from neighbouring local farms. Nonetheless, the investigation was mainly focused on the index farm. As a consequence, it is possible that the problem may be more spatially dispersed than reflected here.

The air dispersion modelling is underpinned by a number of important assumptions, as described in detail in *Appendix 6*. We emphasise that the model assumes an emission rate on a continuous basis (24 hour each day) at maximum permitted levels. In reality, these rates will vary depending on the factory's productivity. Further, the meteorological data is based on synoptic data from Kilkenny City (17 km south of Castlecomer) and high air data from Valentia island (approximately 230 km southwest of Castlecomer). Although the model outputs (as with any model) are a crude (and imperfect) representation of reality, model and observed results were consistent, during preliminary model validation *(Appendix 6)*.

## 4. Conclusion

A number of element deficiencies have been identified in soil, herbage and cattle on the index farm, including Ca, Cu, Se, Zn and, to a lesser extent, I. Similar concentrations have been measured on other farms in the area, where problems of poor animal performance have not been reported. Observed soil conditions (low pH, high liming requirements) are known to adversely affect the availability of essential elements. The condition is not responsive to Se supplementation, consistent with an aetiology more complex than Se alone. A number of animal health concerns were identified on the index farm prior to 2007, with adverse effects on animal performance. Since 2007, however, the potential for such effects has been substantially reduced, following the introduction of robust preventive measures and as a consequence of intensive veterinary supervision by the project team and the farmer's own private veterinary practitioners. There is evidence of background Cd exposure among cattle on the index farm, based on an assessment of Cd kidney in 23 animals during 2003-2005 and 2009. However, these concentrations are not of toxicological significance, based on current knowledge, and would not be expected to adversely affect animal health. Fluoride is present intermittently, but not at concentrations considered detrimental to animal health.

This investigation has sought to address two related questions about this case: the possible cause(s) of the problem and reason(s) why this problem appears to cluster, both in time and space. It is likely that this is a multifactorial condition. A number of factors have been described that undoubtedly have some influence on the performance of cattle on the index farm. However, no comprehensive understanding has yet been established to fully explain the epidemiological presentation of cases, in particular the localisation of cases to the index farm and the temporal distribution of periods when negative performance was recorded. Further work to clarify the spatial extent of the problem, and to provide baseline information about Cd concentrations in Irish cattle at slaughter, may be warranted.

## 5. Acknowledgements

We acknowledge the support and assistance provided by the index farm owner, his family and his private veterinary practitioners to the studies conducted on the index farm. We also thank a range of people who assisted during the compilation of this overview, from the Department of Agriculture, Fisheries and Food (DAFF), University College Dublin (UCD) Veterinary

Sciences Centre, the Environmental Protection Agency (EPA), Teagasc, the Health Service Executive (HSE) and Kilkenny County Council.

## 6. References

### *6.1 Published literature*


Araya, O., Wittner, F., Villa, A. and Ducom, C., 1990. Bovine fluorosis following volcanic activity in the southern Andes. Veterinary Record 126, 641-642.

Asta, J., Erhardt, W., Ferretti, M., Fornasier, F., Kirschbaum, U., Nimis, P.L., Purvis, O.W., Pirintsos, S., Scheidegger, C., Van Haluwyn, C. and Wirth, V., 2002. European guideline for mapping lichen diversity as an indicator of environmental stress . The British Lichen Society, London. At: http://www.thebls.org.uk/content/documents/eumap.pdf. Accessed on 02 December 2008.

Berry, D., Buckley, J., O'Donovan, J., O'Mahony, P. and O'Farrell, K., 2005. Animal health surveillance of dairy herds in the vicinity of a large chemical industrial complex in the Cork harbour region 2001-2004. Cork County Council. At: http://www.corkcoco.ie/co/pdf/129612327.pdf. Accessed on 02 December 2008.

Brodeková, L., Gilmer, A., Dowding, P., Fox, H. and Guttová, A., 2006. An assessment of epiphytic lichen diversity and environmental quality in Knocksink Wood Nature Reserve, Ireland. Biology and Environment: Proceedings of the Royal Irish Academy 106, 215-223.

Buckley, J., O'Donovan, J.V., Berry, D.P., O'Mahony, P. and O'Farrell, K.J., 2007. Comparison of production and calving data for 10 Irish dairy herds in the vicinity of an industrial chemical complex and 10 herds in rural, non-industrialised areas. Veterinary Record 161, 841-845.

Bunce, H.W.F., 1985. Fluorine in air, grass and cattle. Journal of Dairy Science 68, 1706-1711.

Canty, M.J., Lane, E.A., Clegg, T.C., Sharpe, A.E. and More, S.J. A small area study of poor animal performance; results of a selenium supplementation field trial. *[Study 3]*

Canty, M.J., McCormack, S., Lane, E.A., Collins, D.M. and More, S.J. A small area study of poor animal performance; review of trace elements and heavy metals in soil and herbage and their implications for animal health. *[Study 4]*

Canty, M.J., Lane, E.A. and More, S.J. A small area study of poor animal performance; kidney cadmium concentrations in young cattle and cows. *[Study 5]*

Conry, M., Ryan, P., 1967, Soils of Co. Carlow. Soil Survey Bulletin No. 17. An Foras Talúntais.

Cronin, S.J., Manoharan, V., Hedley, M.J. and Loganathan, P., 2000. Fluorine: a review of its fate, bioavailability, and risks of fluorosis in grazed pasture systems in New Zealand. New Zealand Journal of Agricultural Research 43, 295-321.

Elliott, P., Cuzick, J., English, D. and Stern, R., 1992. Geographical and environmental epidemiology. Methods for small area studies. Oxford University Press, Oxford.

Elliott, P., Martuzzi, M. and Shaddick, G., 1995. Spatial statistical methods in environmental epidemiology: a critique. Statistical Methods in Medical Research 4, 137-159.

EPA, 1997. Protocol for the investigative approach to serious animal/human health problems. Environmental Protection Agency, Wexford. At: http://www.epa.ie/downloads/advice/general/protocol_investigation_animal_human_health6.pdf. Accessed on 02 December 2008.

EPA, 2001. Investigations of animal health problems at Askeaton, County Limerick (Main report & summary; Animal health; Soil, herbage feed & water; Human health; Environmental quality). Environmental Protection Agency, Wexford.

EPA, 2004. Final report of Expert Group for Silvermines County Pipperary: lead and other relevant metals. Environmental Protection Agency, Wexford. At: www.epa.ie/downloads/pubs/land. Accessed on 11 February 2009.

European Commission, 1986. Council Directive 1986/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewerage sludge is used in agriculture. Official Journal of the European Union L181, 6-12. 04 July 1986 [including subsequent corrigendum and amendments].

European Commission, 1999. Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition. Official Journal of the European Union L115, 32-46. 04 May 1999 [including subsequent corrigendum and amendments].

European Commission, 2008. Commission Regulation (EC) No 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L173, 6-9. 03 July 2008.

European Food Safety Authority, 2009. EFSA sets lower tolerable intake level for cadmium in food. Press Release, 20 March 2009. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902396263.htm. Accessed on 22 June 2009.

Fay, D., Kramers, G., Zhang, C., McGrath, D. and Grennan, E., 2007. Soil geochemical atlas of Ireland. Teagasc and the Environmental Protection Agency, Ireland

Fleming, G.A., 1978. Trace elements in Irish soils with special reference to cobalt and selenium. PhD thesis, National University of Ireland.

Foihirun, K., Wongwit, W., Kaewkungwal, J., Ramasoota, P. and Sangdee, P., 2006. Preparation of in vivo cow control blood samples for cadmium analysis. The Southeast Asian Journal of Tropical Medicine and Public Health 37, 544-548.

Gardiner, M., Radford, T., 1980, Soil associations of Ireland and their land use potential. Soil Survey Bulletin No. 36. An Foras Talúntais.

Gummow, B., Kirsten, W.F.A., Gummow, R.J. and Heesterbeek, J.A.P., 2006a. A stochastic exposure assessment model to estimate vanadium intake by beef cattle used as sentinels for the South African vanadium mining industry. Preventive Veterinary Medicine 76, 167-184.

Gummow, B., van den Broek, J., Kirsten, W.F.A., Botha, C.J., Noordhuizen, J. P. T. M. and Heesterbeek, J.A.P., 2006b. The assessment of biomarkers in sentinel cattle for monitoring vanadium exposure. Journal of Environmental Monitoring 8, 445-455.

Higgs, K.T. and O'Connor, G., 2005. Stratigraphy and palynology of the Westphalian strata of the Leinster Coalfield, Ireland. Irish Journal of Earth Sciences 23, 65-84.

Hill, A.B., 1965. The environment and disease: association or causation? Proceedings of the Royal Society of Medicine 58: 295-300.

Hillman, D., Bolenbaugh, D.L. and Convey, E.M., 1979. Hypothyroidism and anemia related to fluorine in dairy cattle. Journal of Diary Science 62, 416-423.

Houpert, P., Federspiel, B. and Milhaud, G., 1997. Toxicokinetics of cadmium in lactating and nonlactating ewes after oral and intravenous administration. Environmental Research 72, 140-150.

Houpert, P., Mehennaoui, S., Joseph-Enriquez, B., Federspiel, B. and Milhaud, G., 1995. Pharmacokinetics of cadmium following intravenous and oral administration to non-lactating ewes. Veterinary Research 26, 145-154.

Järup, L., Åkesson, A., 2009. Current status of cadmium as an environmental health problem. Toxicology and Applied Pharmacology [doi:10.1016/j.taap.2009.04.020].

Jeran, Z., Mrak, T., Jaćimović, R., Batič, F., Kastelec, D., Mavsar, R. and Simončič, P., 2007. Epiphytic lichens as biomonitors of atmospheric pollution in Slovenian forests. Environmental Pollution 146, 324-331.

Jubb, T.F., Annand, T.E., Main, D.C. and Murphy, G.M., 1993. Phosphorus supplements and flourosis in cattle - a northern Australian experience. Australian Veterinary Journal 70, 379-383.

Keane, M.G. and Drennan, M.J., 2008. A comparison of Friesian, Aberdeen Angus x Friesian and Belgian Blue x Friesian steers finished at pasture or indoors. Livestock Science 115, 268-278.

Krook, L. and Maylin, G.A., 1979. Industrial fluorine pollution. Chronic fluorine poisoning in Cornwell Island cattle. Cornell Veterinarian 69 Supplement, 1-70.

Kuentzel, W.F., 2002. Closing the mine in Castlecomer: from boom-to-bust or from bust-to-boom? Annual Meeting of the Rural Sociological Society, 16 August 2002, Chicago, IL, USA. At: http://www.uvm.edu/envnr/rm230/ireland/castlecomer_paper.pdf. Accessed on 12 February 2009.

Lane, E.A., Canty, M.J., Clegg, T.A. and More, S.J. A small area study into poor performance on an Irish dairy farm: A randomised controlled study to determine the effect on growth, haematological parameters, serum biochemistry, nutritional and endocrinological status, immune function and disease status in housed weanling cattle between January and May 2007. *[Study 1]*

Lane, E.A., Canty, M.J., Clegg, T.A. and More, S.J. A small area study into poor performance on an Irish dairy farm: An investigation to critically evaluate performance and the underlying mechanisms of performance (including nutrition and metabolism) in ten Aberdeen Angus cross Friesian cattle kept on the index farm between January 2007 and April 2008. *[Study 2]*

Lane, E.A., Canty, M.J. Consequences of Cd exposure in cattle: a review**.** *(Overview, Appendix 8)*

Loganathan, P., Hedley, M.J., Grace, N.D., Lee, J., Cronin, S.J., Bolan, N.S. and Zanders, J.M., 2003. Fertiliser contaminants in New Zealand grazed pasture with special reference to cadmium and fluorine - a review. Australian Journal of Soil Research 41, 501-532.

López Alonso M., Benedito, J.L., Miranda, M., Castillo, C., Hernández, J. and Shore, R.F., 2000. Arsenic, cadmium, lead, copper and zinc in cattle from Galicia, NW Spain. The Science of the Total Environment 246, 237-248.

Lynch, G.P., Smith, D.F., Fisher, M., Pike, T.L. and Weinland, B.T., 1976. Physiological responses of calves to cadmium and lead. Journal of Animal Science 42, 410-421.

Magyar, M.I., Mitchell, V.G., Ladson, A.R., Diaper, C., 2008. Lead and other heavy metals: common contaminants of rainwater tanks in Melbourne. Water Down Under, 14-17 April 2008, Adelaide, Australia. pp. 409-417.

Maylin, G.A., Eckerlin, R.A. and Krook, L., 1987. Fluorine intoxication in dairy calves. Cornell Veterinarian 77, 84-98.

McGrath, D., McCormack, R.J., 1999. The significance of heavy metals and organic micropollutants in soils. End of project report. ARMIS 4268 (Teagasc, Johnstown Castle Research Centre, Wexford, Ireland).

McGrath, D., Zhang, C. and Carton, O.T., 2004. Geostatistical analyses and hazard assessment on soil lead in Silvermines area, Ireland. Environmental Pollution 127, 239-248.

McLaughlin, M.J., Tiller, K.G., Naidu, R. and Stevens, D.P., 1996. Review: the behaviour and environmental impact of contaminants in fertilizers. Australian Journal of Soil Research 34, 1-54.

Miranda, M., López Alonso, M., Castillo, C., Hernández, J., Benedito, J.L., 2001, Cadmium levels in liver, kidney and meat in claves from Austrias (North Spain). European Food Research and Technology 212, 426-430.

Moriarty, J.P., Toolan, D.P., Casey, M.J., Barry, J. and McLaughlin, J.G., 2007. Stunting in cattle on three farms associated with calfhood disease. Irish Veterinary Journal 60, 742-746.

National Research Council, 2001. Nutrient requirements of dairy cattle, 7$^{th}$ revised edition. The National Academies Press, Washington.

Neathery, M.W. and Miller, W.J., 1975. Metabolism and toxicity of cadmium, mercury, and lead in animals: a review. Journal of Dairy Science 58, 1767-1781.

Oetzel, G.R., 2007. Subacute ruminal acidosis in dairy herds: physiology, pathophysiology, milk fat responses, and nutritional management. 40th Annual Conference, American Association of Bovine Practitioners, 17 September 2007, Vancouver, BC, Canada. pp. 89-119.

Patra R.C., Swarup, D., Naresh, R., Kumar, P., Nandi, D., Shekhar, P., Roy, S. and Ali, S.L., 2007. Tail hair as an indicator of environmental exposure of cows to lead and cadmium in different industrial areas. Ecotoxicology and Environmental Safety 66, 127-131.

Phillips, C.V. and Goodman, K.J., 2004. The missed lessons of Sir Austin Bradford Hill. Epidemiologic Perspectives and Innovation 1: 3.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D., 2007. Veterinary Medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats, 10th edition. Saunders Elsevier, Edinburgh.

Real, C., Aboal, C.R., Fernández, J.A. and Carballeira, A., 2003. The use of native mosses to monitor fluorine levels—and associated temporal variations—in the vicinity of an aluminium smelter. Atmospheric Environment 37, 3091-3102.

Rothman, K.J., Greenland, S., Poole, C. and Lash, T.L., 2008. Causation and causal inference. In: Rothman, K.J., Greenland, S. and Lash, T.L. Modern Epidemiology, 3rd edition. Wolters Kluwer, Lippincott Williams & Wilkins, Philadephia. pp.5-31.

Scott, H.M., Soskolne, C.L., Martin, S.W., Basarab, J.A., Coppock, R.W., Guidotti, T.L. and Lissemore, K.D., 2003a. Lack of associations between air emissions from sour-gas processing plants and beef cow–calf herd health and productivity in Alberta, Canada. Preventive Veterinary Medicine 57, 35-68.

Scott, H.M., Soskolne, C.L., Martin, S.W., Shoukri, M.M., Lissemore, K.D., Coppock, R.W. and Guidotti, T.L., 2003b. Air emissions from sour-gas processing plants and dairy-cattle reproduction in Alberta, Canada. Preventive Veterinary Medicine 57, 69-95.

Sidle, C., Tuckett-Jones, B., Ng, B and Shi, J.P., 2004. Model intercomparison between ADMS 3.1, AERMOD and AERMOD PRIME. 9th International Conference on Harmonisation within Atmospheric Dispersion Modelling for Regulatory Purposes. Garmisch-Partenkirchen, Germany, 1-4 June 2004. pp. 156-160.

Suttie, J.W., 1980. Nutritional aspects of fluorine toxicosis. Journal of Animal Science 51, 759-766.

Swarup, D., Naresh, R., Varshney, V.P., Balagangatharathilagar, M., Kumar, P., Nandi, D. and Patra, R.C., 2007. Changes in plasma hormones profile and liver function in cows naturally exposed to lead and cadmium around different industrial areas. Research in Veterinary Science 82, 16-21.

US EPA, 1997. Compilation of Air Pollutant Emission Factors, Volume I: Stationary Point and Area Sources [AP42]. Chapter 11.3 Bricks and Related Clay Products. United States Environmental Protection Agency. http://www.epa.gov/ttn/chief/ap42/ch11/index.html. Accessed on 04 December 2008.

US EPA, 2003. National Emission Standards for Hazardous Air Pollutants for Brick and Structural Clay Products Manufacturing; and National Emission Standards for Hazardous Air Pollutants for Clay Ceramics Manufacturing; Final Rule [40 CFR Part 63]. United States Environmental Protection Agency. Federal Register vol. 68 no. 5 (16

May 2003), pp 26690-26755. At: http://www.epa.gov/ttn/atw/brick/fr16my03.pdf. Accessed on 02 December 2008.

Waldner, C., 2001. Monitoring beef cattle productivity as a measure of environmental health. Environmental Research 86, 94-106.

Waldner, C.L., Ribble, C.S., Janzen, E.D. and Campbell, J.R., 2001. Associations between total sulfation, hydrogen sulfide deposition, and beef-cattle breeding outcomes in western Canada. Preventive Veterinary Medicine 50, 19-33.

Weinstein, L.H. and Davison, A.W., 2003. Native plant species suitable as bioindicators and biomonitors for airborne fluorine, Environmental Pollution 125, 3-11.

Weiss, C. (2003a) Scientific uncertainty and science-based precaution. International Environmental Agreements: Politics, Law and Economics 3, 137–166.

Weiss, C. (2003b) Expressing scientific uncertainty. Law, Probability and Risk 2, 25-46.

World Bank Group, 1999. Pollution prevention and abatement handbook 1998: Toward cleaner production. The World Bank Group in collaboration with the United Nations Development Programme and the United Nations Industrial Development Organization. The World Bank Group, Washington.

### *6.2 Unpublished reports*

Cody et al., 2005. Teagasc, Kilkenny.

Crilly, 2004. Results of trace element levels in samples taken from the index farm. Teagasc, 21 October 2004.

DAFF, 2006. Investigation of animal health problems on a farm in Castlecomer, Co. Kilkenny [Volumes I and II, with supporting documentation]. Veterinary Laboratory Service, Department of Agriculture and Food, 7 June 2006.

EPA, 2006. Summary report of the work completed by the EPA as part of the multi-agency investigation of animal health in Castlecomer, Co. Kilkenny. Environmental Protection Agency, September 2006.

Gardiner, J.J., 2005. Condition of trees on the farm of Dan Brennan. Drumgoole, Castlecomer, Co. Kilkenny.

Gardiner, J.J., 2007. Analysis of foliage samples from trees on Mr Brennan's farm and of soil samples taken on 23 August 2007.

Kavanagh, S. and Cody, M., 2004. Nutritional status of the farm. Teagasc, March 2004.

More, S.J., 2005. The Castlecomer investigation: an epidemiological review. University College Dublin, 29 March 2005.

UCC, 2006. An impact assessment of epiphytic lichens. Aquatic Services Unit, Environmental Research Institute, University College Cork

White, S., 2005. Report on observations of hedgerows on the farm of Mr Dan Brennan, Castlecomer, Co. Kilkenny. Simon White, Environmental Scientist, 2 September 2005

White, S., 2009. Follow-on report on observations of hedgerows on the farm of Mr Dan Brennan, Castlecomer, Co. Kilkenny. Simon White, Environmental Scientist, 20 February 2009.