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Managing the contaminants in feedlot wastes: Development of realistic guidelines – Summary Report

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Glossary

Term/Concept/ Abbreviation	Explanation/ Full Wording
"Hazardous events" and "sources of hazards"	Those incidents or situations that can contribute to the presence of a hazard (what can happen and how). This may include point sources of pollution such as human and industrial waste ..... as well as diffuse sources such as those arising from agricultural and animal husbandry activities. Other examples include continuous, intermittent or seasonal (contamination) patterns as well as extreme and infrequent events. Nadebaum et al. (2004)
ABARE	Australian Bureau of Agricultural Research and Resource Economics
ACGIH	American Conference of Governmental Industrial Hygienists (USA)
ADI	Acceptable Daily Intake
ADWG	Australian Drinking Water Guidelines
Autochthonous	The indigenous flora and fauna of a region (includes microorganisms).
BRD	Bovine Respiratory Disease, also known as 'Shipping fever', is one of the most common diseases in feedlots.
CAFO	Concentrated Animal Feeding Operation (USA)
COPC	Chemicals of Potential Concern
Dander	Particles of dry scales or fluff shed from the skin, hair or feathers of animals, which may act as allergens
EDC	Endocrine Disrupting Compound
EHEC_EPEC	Enterohaemorrhagic *E. coli* _ Enteropathogenic *E. coli* – subgroup of *E. coli* detected through qPCR
Endotoxin	is a lipopolysaccharide (LPS) component of the outer cell wall of Gram-negative bacteria. Endotoxin is a potent inflammatory agent that produces systemic effects and lung obstruction, even at low levels of exposure. Livestock confinement units present some of the highest concentrations seen anywhere.
Etiology	The study of the cause of a disease
FHP	A major disease caused by aspergilli found in mouldy hay, straw and feed is "farmer's hypersensitivity pneumonitis (FHP), previously referred to as farmer's lung disease.
FISH	Laboratory inactivation studies have been undertaken in Australian soils at 20 ºC and 35 ºC using fluorescent *in situ* hybridization (FISH) as a conservative measure of *C. parvum* oocyst viability.
FSA	Feedlot Services Australia
Fulminant	Developing or progressing suddenly, severely and rapidly
GC-MS/MS	Gas Chromatography Double Mass Spectrometry.
GFP	Green Fluorescent Protein (label for inoculated bacteria)
HACCP	Hazard Analysis and Critical Control Points
Hazard	A biological, chemical or physical agent in, or condition of, food (or waste) with the potential to cause an adverse health effect. Codex Alimentarius (1999)
Hazard identification	The identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods. Nadebaum et al. (2004)
HC	haemorrhagic colitis – concern with EHECs and STECs
HGPs	hormonal growth promotants such as Trenbolone
HPLC-MS/MS	High Pressure Liquid Chromatography followed by double Mass Spectrometry.
HQ	Hazard Quotient (ratio of expected level of exposure to a chemical and the safe level of exposure)
HRA	Health Risk Assessment
HUS	haemolytic uraemic syndrome – concern with EHECs and STECs
IBR	Infectious Bovine Rhinotracheitis
Metamodel	A model framework composted of sub models/modules which can be varied according to the modelling aims.
MLA	Meat and Livestock Australia Limits
MPN	Most probable number (measure of levels of bacteria detected by tube/broth growth medium.
Mycotoxin	Toxins produced by fungi – typically secondary metabolites. Ergot on peanuts is an example of a fungus forming such toxins (see review for more details).

Term/Concept/ Abbreviation	Explanation/ Full Wording
NASAA	National Association of Sustainable Agriculture in Australia.
NFAS	National Feedlot Accreditation Scheme
ng	Nanogram
NH&MRC	National Health and Medical Research Council
NIOSH	The National Institute for Occupational Safety and Health (USA)
NOEL	No Observed Effects Level
ODTS	Organic Dust Toxicity Syndrome (illness sometimes seen after exposure (inhalation) of high levels of aerosols from intense animal rearing operations.
OSHA	Occupational Safety and Health Administration (USA)
Pathogen	Microorganism that causes disease. *Salmonella enterica* is a well-known example.
Pathogenic and Toxigenic *E. coli*	There are many strains of toxin producing or otherwise pathogenic *E. coli* . A reviews of the state of knowledge can be found at Kaper et al. (2004) and Muniesa et al. (2006).
PDF	Probability Density Function - In probability theory, a probability density function (pdf), or density of a continuous random variable is a function that describes the relative likelihood for this random variable to occur at a given point. The probability for the random variable to fall within a particular region is given by the integral of this variable's density over the region. (Wikipedia)
PEPA	Preliminary Exposure Pathway Assessment – use to describe the process by which high priority exposure pathways were identified
Phenotype	Observable or physical manifestation of an organism
Plates (Assay Method)	Small dishes filled with a nutrient agar formulated to grow selected microorganisms such as bacterial indicators.
PM_{10} and $PM_{2.5}$	PM_{10} refers to particulate matter less than 10 µm in diameter and $PM_{2.5}$ is less than 2.5 µm in diameter. In general, finer particulate fractions contain a higher proportion of anthropogenic dust and lower levels of wind-blown soil and plant pollens. Since lung problems associated with CAFOs include airway disease, it is important to consider inhalable particulate fraction and PM_{10}.
PrP	prion protein
QCRA	Quantitative Chemical Risk Assessment
QMRA	Quantitative Microbial Risk Assessment
qPCR	Quantitative Polymerase Chain reaction technology used to quantify the numbers of a selected gene sequence in a sample and infer the numbers of organisms present containing that gene sequence.
QRA	Quantitative Risk Assessment (Generally)
QUT	Queensland Institute of Technology
Risk	The probability that, in a certain timeframe, an adverse outcome will occur in a person, group of people, plants, animals and/or the ecology of a specified area that is exposed to a particular dose or concentration of a hazardous agent, i.e. it depends on both the level of toxicity of the agent and the level of exposure (Department of Health and Aging, 2002).
Risk assessment	A scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterisation, (iii) exposure assessment, and (iv) risk characterisation. Codex Alimentarius (1999)
Risk management	refers to the overall process of evaluating the ..... system, identifying hazards, sources and hazardous events, assessing and prioritising risks, and developing and implementing effective preventive measures and strategies to manage the risks. Nadebaum et al. (2004)
RSD	Relative Standard Deviation (SD/mean usually expressed as a percentage)
SD	Standard Deviation
Sequelae	Morbid conditions resulting from a previous disease
Seroprevalence	Number of individuals that test positive for a particular disease
Serovar/ serotype	Subdivision of microorganisms based on antigens
STEC	Shigatoxin producing *E. coli.* Some are recognized as human pathogens causing severe diseases
Synoptic Survey	Also known as a reconnaissance survey. Describes a group of observations that give a broad view of a subject at a particular time.

Term/Concept/ Abbreviation	Explanation/ Full Wording
Taxa	A taxon (plural: taxa) is a group of (one or more) organisms, which a taxonomist adjudges to be a unit. e.g. *Bos taurus*.
TSEs	transmissible spongiform encephalopathies - not reported in Australian cattle, they were highlighted in the UK in 1986, where bovine spongiform encephalopathy (BSE)
UF	Uncertainty factor
VTEC	verocytotoxigenic *E. coli* . Verotoxins can damage renal and endothelial cells.
WHO	World Health Organization
WRC	University of NSW Water Research Centre

Executive Summary

The Risk Assessment and Management Process

FLOT Project 333 has undertaken an assessment of the risks from pathogens and chemicals in manures to exposed human populations and explored their fate and transport with a view to understanding the form and scale of risks and developing risk management recommendations for MLA reflecting this understanding. It has done this by:

1. Measuring the levels of contaminants within major/priority manure waste-streams at operational feedlots;
2. Conceptualizing the hazards and the exposure pathways;
3. Combining this information with dose response literature;
4. Using risk models, which integrate this information, characterized the absolute and relative risks arising under a range of representative exposure scenarios;
5. Developing management recommendations designed to minimize risks consistent with the emerging exposure picture and current manner in which feedlots are operated.

The exposure scenarios modelled reflect a review of the literature, the data collected on feedlot contaminant levels and their inactivation/decomposition during management, visits to feedlots to understand current waste management practice, a provisional exposure pathway assessment and conservative/balanced selection of input assumptions in the risk models constructed.

The foci of the assessment were:

1. Major feedlot waste streams likely to contain high loads of zoonotic pathogens and chemical contaminants:
    - fresh faeces;
    - pen manure;
    - harvested manure;
    - aged manure;
    - composted manure;
    - carcass compost; and
    - (secondarily) site run-off;
2. Priority contaminants identified in the initial literature review and via discussions with lot feeders as to their current operation practice comprised:
    - 10 zoonotic pathogens;
    - 5 bacterial indicators (not necessarily hazardous but having lifecycles indicative of pathogens);
    - 13 endocrine disrupting compounds (steroidal hormones);
    - 4 parasiticides;
3. Risks arising from aerosol and dust exposure (inhalation and ingestion) to the following populations:
    - On-farm workers;
    - On-farm visitors;
    - Off-farm users of waste products;
    - The public in situations where exposure appears most likely.

The work program undertaken to underpin the risk assessment included the following activities:

1. A literature review to prioritize experiments and identify appropriate/logistically feasible assay techniques and other experimental methods:
2. Contaminant assay development and adaptation in particular:
    a. Development of quantitative Polymerase Chain Reaction (qPCR) assays for measuring the abundance of pathogens and microbial indicators in wastes;
    b. Adaptation of established microbial culture assays used for indicators.
    c. Development of extraction methods and assays for the key trace organic compounds of concern.
3. A survey of contaminant levels in major wastes:

   a. At 5 feedlots (3 in Queensland, 1 in NSW and 1 in Victoria);
   b. During two seasons (winter and summer);
4. Measurement of the rate of inactivation (pathogens) or decomposition/disappearance (chemicals):
   a. in manures as a function of temperature(20, 37, 50 and 60 ^{o}C), over time (up to 4 months);
   b. in response to exposure to solar radiation (short term disinfection only);
   c. *in situ*(background levels); in microcosms (background levels); in microcosms (inoculated model microorganisms);
   d. in run-off ponds;
5. Characterization of aerosols generated at feedlots during a relatively dry period (2 Feedlots, late spring 2009):
   a. measured at the centre of one feedlot virtually continuously over 4 days (treated as ambient particle content);
   b. measured immediately downwind of 29 different activities at 2 Feedlots generating aerosols on a small, medium and large scale.

The management recommendations developed are based on a combination of hygiene first principles, risk probability estimates based on the new data outlined above, and discussion/ observations/inspection of Feedlot operations.

Figure A shows how the assessment actions follow and relate to one another. The key activities outlined in this figure have been used as a guide to structure this report.

Figure B summarizes the main exposure pathways characterized and illustrates application of risk assessment concepts.

Figure C outlines the risk characterization process.

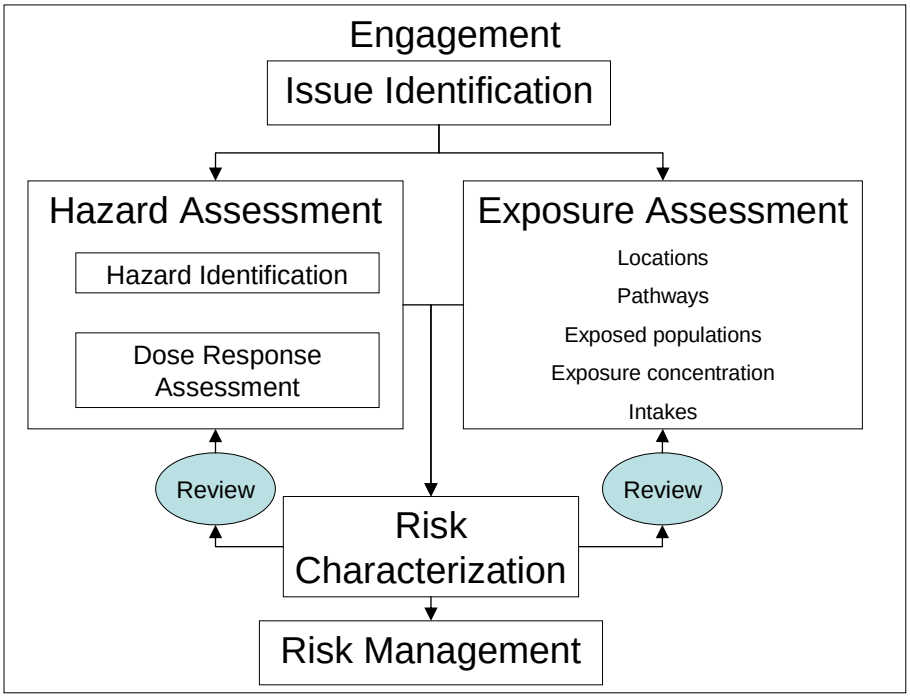

Figure A. Summary of the Health Risk Assessment Process Recommended by WRC/FSA for Feedlots

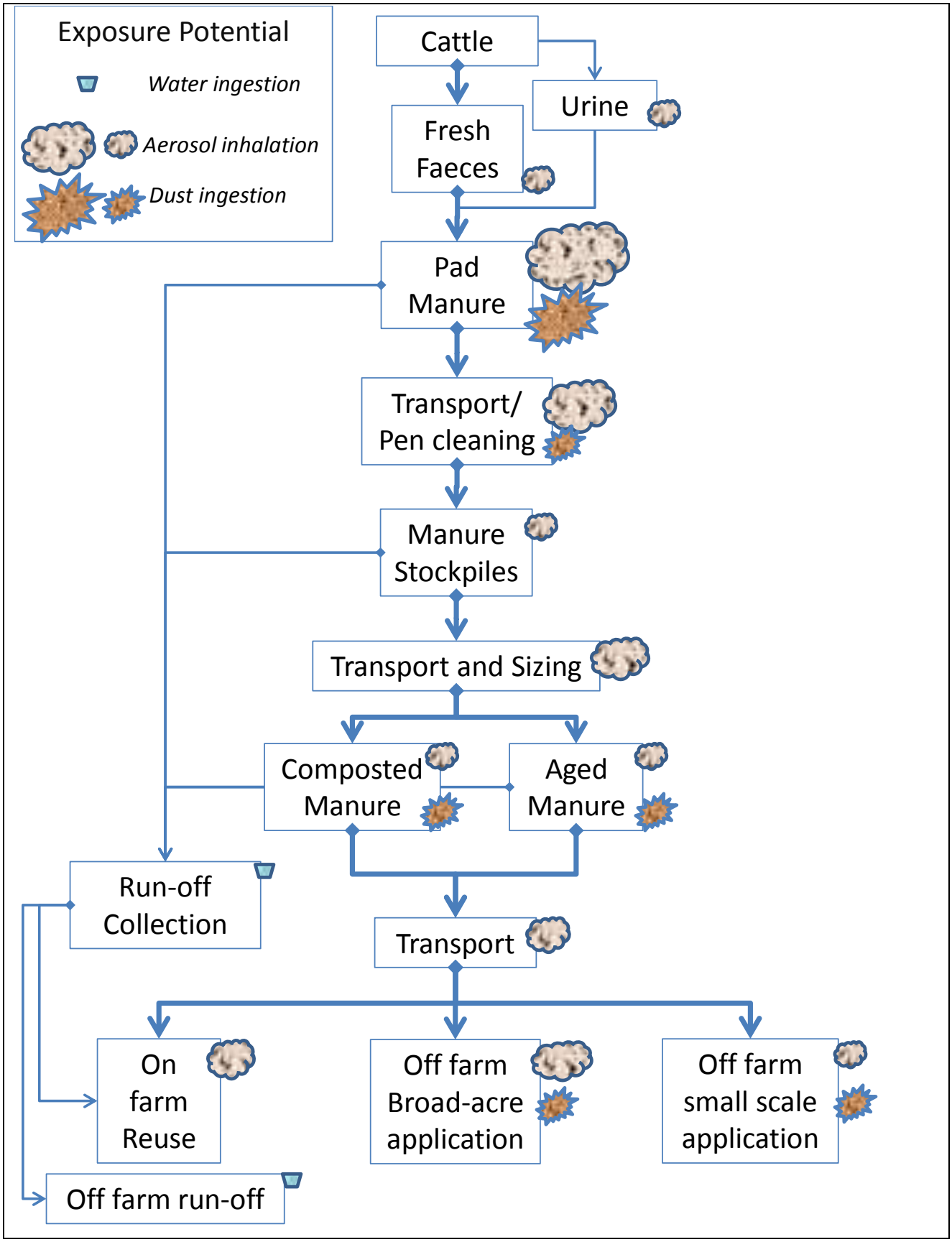

Figure B. Exposure pathways showing locations of manure contaminants where exposure is likely and how they are linked to one another.

a. Relative potential exposure is illustrated by the size of the icons
b. Large arrows indicate the main contaminant transport pathways.

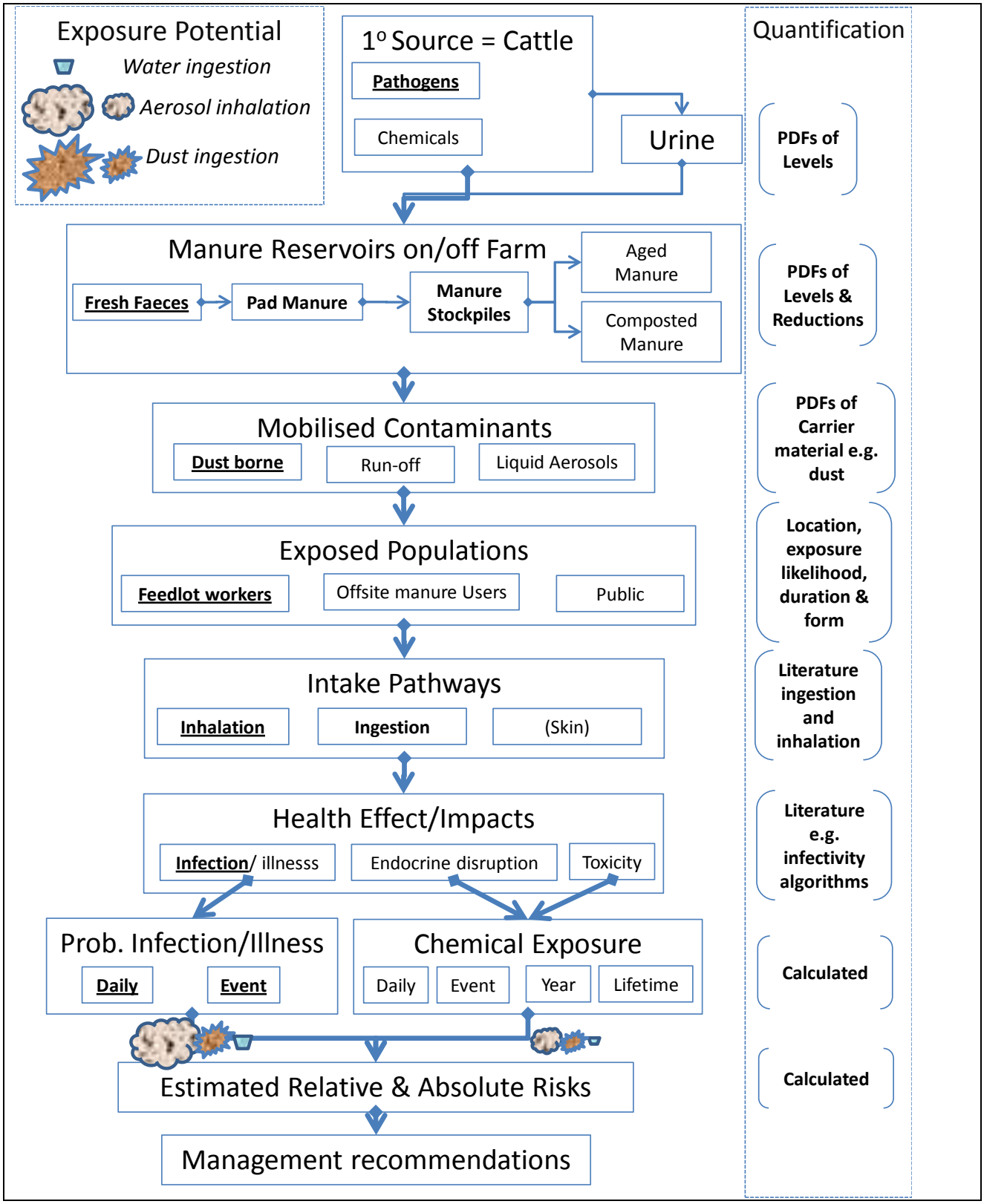

Figure C. Generic Risk Characterization Process
a. Relative potential exposure is illustrated by the size of the icons
b. Bold and underlining emphasize highest concerns

Risk Characterization Outcomes

All pathogens (and indicators) assayed for were detected, many in substantial numbers.

Chemicals were by in large present in low concentrations or absent.

Generally, pathogen exposure poses a greater risk than chemicals. However, pathogens are generally more labile than chemicals so there is significant opportunity for reducing their numbers to tolerable levels.

The levels of many contaminants observed in different manures appeared consistent with literature reports, most notably the abundance of EHEC_EPEC *E. coli* and *Campylobacter.*

Exposure pathways involving contaminated aerosols were confirmed as posing relatively high risks, particularly pen manure aerosols. Generic dust ingestion was also identified as a potential problem.

Quantitative risk characterization is still at an early development stage for water management, the model for the current approach, and in the case of Feedlot wastes development is clearly even less developed despite the large amount of scientific data collected in the USA and Europe. However the quantitative and qualitative approaches and benchmarks developed for the water industry appeared applicable.

The (Provisional) risk characterization outcomes are summarized in Table I. Overall varying levels of pathogen management and exposure minimization appear necessary,. These should also reduce residual concerns over chemical contaminants as well.

Table I Summary of Characterized Pathogen Risks

Issue	Findings and Observations	Conclusions
On-farm Pad Manure Dust inhalation	• The high dust levels measured combined with the high pathogen content of pad manure assessed in the survey to generate a number of *High* risk ratings. • Relatively *High* ratings were seen not only with site workers but also visitor exposure. • *High* risk ratings were also estimated when low and intermediate dust levels were assumed.	• Recognizing that pen manure dust aerosol generation is probably inevitable at any feedlot during dry weather, active management of exposure to pen manure dust is still probably needed. • Generic options include: o avoidance, especially of the evening peak; o hygiene education; o making protective devices available; o wetting of pad surfaces • Management actions should be targeted at all feedlot workers and visitors.
On-farm Dust Ingestion	• As with inhalation several pathogen in pen manure appear to pose a *High* risk. • Working with Aged manure for an extended period of time also appears to pose a relatively *High* risk. • Robust composting sufficient to reduce pathogen numbers by 5 orders of magnitude reduces risk ratings to a *Low* to *Very Low*.	• Pen manure should be actively managed. • Aged manure can still contain a range of pathogens at levels of concern which require management. Export off-site needs to consider how exposure to downstream users should be controlled. Simply aging manure prior to export may not be sufficient. • Composting or equivalent effective pasteurization should be able to achieve a *Low* to *Very Low risk* rating even in the event of exposure over an extended period of time.
Inhalation of dust during Small Scale Activities On-farm	• All risk ratings were estimated to be *Low* or *Very Low.* • The exposure conditions explored were worst case i.e. immediately and closely downwind of the activity modelled.	• Transient exposure to small dust plume events of duration 10 seconds to a few minutes appears to pose a relatively *Low* risk (mainly aged manure and composted manure).

Issue	Findings and Observations	Conclusions
Inhalation of dust during Medium to Large Scale Activities On-farm	• There was no clear difference between events classed as medium and large so the two are considered here together. • Risk ratings for Aged manure and composted manure were generally estimated to be *Low* or *Very Low.* • However some ratings for harvested manure were estimated to be *Moderate* to *High*. • The exposure conditions explored were worst case i.e. immediate and intimate exposure downwind of the activities modelled.	• Transient exposure to dust plume events when managing harvested manure appears to require active management comparable to that for pen manure dust management (avoidance, education etc.).
Off-farm dust ingestion	• Working with Aged manure for an extended period of time also appears to pose a relatively *High* risk. • But robust composting sufficient to reduce pathogen numbers by 5 orders of magnitude reduce risk ratings to a *Low* to *Very Low*.	• Aged manure can still contain a range of pathogens at levels of concern which require management and export off-site needs to consider how exposure to them should be subsequently controlled. • Composting or equivalent effective pasteurization should be able to achieve a *Low* to *Very Low risk* rating even in the event of exposure over an extended period of time.
Off-farm Inhalation of Small Quantities of Manure and Compost Dust During Events	• The risks considered were assessed as being *Low* to *Very Low*.	• Short term exposures were assessed as having short term risk ratings of *Low* to *Very Low*.
Inhalation of Dust During Aged Manure and Compost Transport	• Transporting compost appeared to pose *Very Low* risk from short duration fugitive emissions. • Transporting aged manure posed a *Low* to *Very Low* risk unless exposure was both extended and the aerosol concentration was high.	• During transport aged manure and composted manure should be covered. The occasional fugitive emission does not appear to pose a substantial risk. • This may not be applicable to fresh pen manure and its early transport off-site is not recommended.

Management Recommendations

Based on the characterized risks, recommendations for minimising exposure have been drafted in guidelines format with producer waste management in mind (Appendix 37 Guidelines for the Safe Management of Feedlot Wastes).

These should be seen as provisional Guidelines at this stage bearing in mind the first task of risk assessment is to engage with all stakeholders. As with environmental and other management systems these are seen as living documents to be modified and adapted firstly in light of the operating conditions of feedlots, secondly in light of new information as it emerges in the future and thirdly future stakeholder discussions and feedback.

The following are the primary recommendations regarding management.

- Risk management recommendations should be applicable to all feedlots irrespective of state or geographic locality.
- Risk management recommendations are directed at protecting against pathogen risks except where indicated.
- MLA should promote/establish systems to ensure:

    - awareness that manure, pen manure in particular, has significant numbers of all 10 zoonotic pathogens surveyed including the EHEC_EPEC group, *Campylobacter,* and *Cryptosporidium*;
    - awareness that the pathogens pose a range of risks, in the first place gastroenteritis (e.g. EHEC_EPEC, *Campylobacter*, *Giardia*), but also can cause other diseases/sequelae (Q Fever, Leptospirosis);
    - awareness that processed manure wastes contain reduced but still significant numbers of pathogens and material must be treated accordingly;
    - good hygiene among all staff especially pen workers and other outdoor staff;
- It is suggested that groups at relatively higher risk (e.g. elderly , immunocompromised) should be advised to minimize their exposure to the open air Feedlot environment (e.g. women should be advised of the high numbers of *Listeria monocytogenes* in the pen manure even though their infectivity was judged to be low).
- Procedures developed in the water industry for handling and recycling biosolids safely appear in principle be appropriate for application to manure.
- Fine aerosolized dust generated by wind, cattle and feedlot activities during dry periods appear to pose the greatest risk.
- During dry periods consideration might be given to wetting / dust suppression of the pad manure.
- The use of run-off water is not recommended for dust suppression without treatment . Run-off water is initial very highly contaminated. More work is probably needed to optimise its management and harmonise this with local hydrological regimes.

A range of detailed recommendations are also included which cover:

- Protection of On-farm Staff
- Manure Management On-site
- Irrigation Runoff Water
- On-farm Visitors
- Off-farm Manure Reuse/Users
- The Public
- Composting and Aging
- Composted Carcasses
- Major Reuse situations
- Monitoring of Aged Manure and Composted Manure Quality

Conclusions

- Feedlot manure wastes pose a human health risk primarily from zoonotic pathogens under certain exposure conditions rather than chemical residuals.
- The primary material of concern is pen manure.
- The primary exposure pathways of concern are those involving aerosols.
- The risks can probably be managed on-site and have probably have already been mitigated by current best practice management e.g. stormwater collection basins.
- Widespread safe reuse appears viable which could add greatly to the value of the waste material provided contaminant management is implemented to the appropriate levels discussed above.

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I Background

I.1 Scope of Feedlot Studies – Health Risk Assessment of Feedlot Contaminants

I.1.1 What FLOT.333 involved and what it was designed to achieve

FLOT Project 333 has undertaken an assessment of the risks from pathogens and chemicals in manures to exposed human populations in Australia and explored their fate and transport with a view to understanding the form and scale of risks and developing risk management recommendations for MLA stakeholders. It has done this through the application of established risk assessment and management principles. Specifically it has:

1. Identified contaminants within major manure waste-streams at operational feedlots and estimated their levels through analysis of material from representative feedlots;
2. Conceptualized the hazards and the exposure pathways by which different populations might inhale or ingest priority contaminants through feedlot inspections and user analysis;
3. Combined this information with dose response literature;
4. Using risk modelling, integrated this information, and estimated relative, and to a degree absolute, risks arising under a range of exposure scenarios;
5. Developed management recommendations designed to minimize risks arising in various exposure scenarios consistent with the current manner in which feedlots are operated and waste is managed.

The exposure scenarios modelled reflect a review of the literature, user interactions, the data collected on feedlot contaminant levels and their inactivation/decomposition during management, visits to feedlots to understand current waste management practice, a provisional exposure pathway assessment and conservative/balanced selection of input assumptions in the risk models constructed.

Specifically the assessment focused on:

1. Major feedlot waste streams likely to contain high loads of zoonotic pathogens and chemical contaminants:
    - fresh faeces;
    - pen manure;
    - harvested manure;
    - aged manure;
    - composted manure;
    - carcass compost; and
    - (secondarily) site run-off;
2. Priority contaminants identified in the initial literature review and via experimental surveys of lot feeders as to their current operation practice comprised:
    - 10 zoonotic pathogens;
    - 5 bacterial indicators (not necessarily hazardous but having lifecycles indicative of pathogens);
    - 13 steroidal endocrine disrupting compounds(EDCs);
    - 4 parasiticides;
3. Risks arising from aerosol and dust exposure (inhalation and ingestion) to the following populations:
    - On-farm workers;
    - On-farm visitors;
    - Off-farm users of waste products;
    - The public in sporadic situations where some brief exposure appeared possible.

Operationally the risk assessment undertook the following activities:

1. Questionnaire based surveys of current waste management and reuse practice;
2. Literature review of:

   a. possible contaminants, the risk they posed and their management, to prioritize experiments and identify appropriate/logistically feasible assay techniques and other experimental methods;
   b. carcass composting.
3. Assay development and adaptation in particular:
   a. Development of quantitative Polymerase Chain Reaction (qPCR) assays for measuring the abundance of pathogens and microbial indicators in wastes;
   b. Adaptation of established microbial culture assays for quantifying indicators;
   c. Development of extraction methods and assays for EDCs and ectoparasiticides of potential concern.
4. A survey of contaminant levels in major wastes:
   a. At 5 feedlots (3 in Queensland, 1 in NSW and 1 in Victoria);
   b. During two seasons (winter and summer);
5. Measurement of the rate of inactivation (pathogens) or decomposition/disappearance (chemicals):
   a. in manures as a function of temperature (20, 37, 50 and 60 ^{o}C), over time (up to 4 months);
   b. in response to exposure to solar radiation (short term disinfection only);
   c. *in situ*(background levels); in microcosms (background levels); in microcosms (inoculated model microorganisms);
   d. in run-off ponds (indicators only);
6. Characterization of aerosol generation at feedlots during a relatively dry period (2 Feedlots, late spring 2009) which involved measurement of;
   a. ambient concentrations at the centre of one feedlot during a dry period virtually continuously over 4 days (treated as ambient particle content);
   b. transient aerosol concentrations immediately downwind of 29 different actual and simulated manure management activities (small, medium and large scale) at 2 Feedlots.

The management recommendations developed are based on a combination of hygiene first principles, risk probability estimates based on the new data outlined above, and discussion/ observations/ inspection of Feedlot operations. Most of the data and analyses have been compiled in stand-alone Appendices (Table 0-1) which are also cross-referenced at appropriate points within the main report body.

Table 0-1. List of Appendices

Appendix No.	Appendix Title
1	End-user Analysis
2	Survey of Existing Waste Management Practices at Model Feedlots
3	Composters Case Study
4	Experimental Project Plan Summary for Feedlot Co-Operators
5	Review of Contaminants in Feedlot Wastes
6	Carcass Composting Review
7	Preliminary Assessment of Risk and Refinement of FLOT.333 Project Plan
8	Aerosol Measurement Campaign Plan
9	Interim Results Report
10	Monitoring Bacterial Indicators and Pathogens in Cattle Feedlot Waste by Real-Time PCR
11	Ectoparasiticides in Australian Beef Cattle Feedlot Wastes
12	Estrogens, Androgens and Progesterone in Solid Waste
13	Pathogens and Indicators in Cattle Feedlot Manure –Primary Survey Results
14	Indicator Monitoring Reliability
15	Supplementary Survey Data on Pathogens in Feedlot Wastes
16	Pathogenic Leptospira - False Positive Detection, Improvement of Quantification Method and Reanalysis of Feedlot Wastes
17	Pathogen Dose Response
18	Chemical Dose Response Algorithms
19	Inactivation Kinetics of Model Microorganisms

Appendix No.	Appendix Title
20	Inactivation Rate Kinetics Temperature Dependency
21	Inactivation of Bacteria in Cattle Manure Dust in the Dark and by Solar Radiation
22	Dust Measurements at Cattle Feedlots
23	Feedlot Dust Emission Photographs
24	Particle Emission Characteristics
25	Event Based Aerosol Particle Loadings
26	Feedlot Run-off Bacteriological Quality during Significant Storms
27	Dust Emission PDFs
28	Ingestion and Inhalation
29	Exposure Scenarios
30	Risk Benchmarking Considerations
31	FLOT 333 Risk Benchmarking
32	Chemical Risk Benchmarking
33	Primary Input Assumptions for Estimation of Risk
34	Pathogen Risk Rating Tables
35	Pathogen Risk Ratings for Aerosol Inhalation and Dust Ingestion Scenarios
36	Chemical Hazard Ratings (-log10 HQ) for Steroidal Hormones and Ectoparasiticides
37	Guidelines for the Safe Management of Feedlot Wastes

I.1.2 Risk Assessment Framework

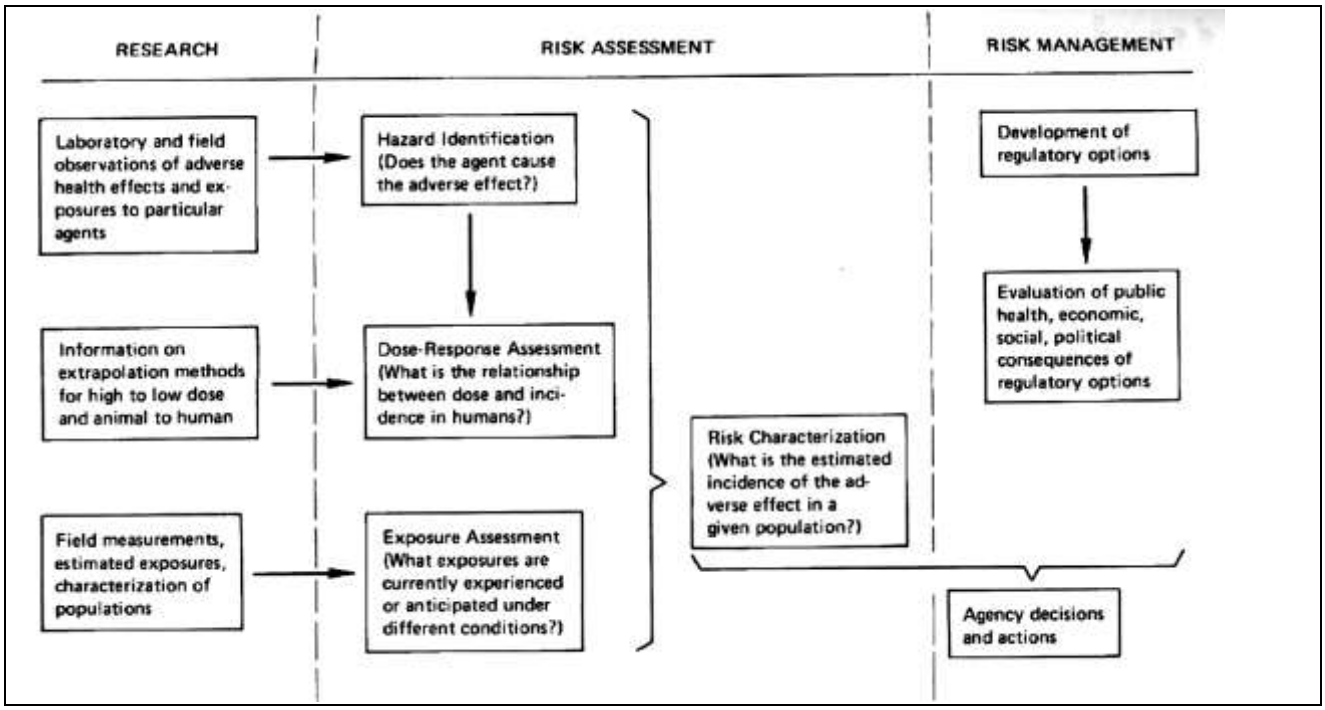


Figure 0-1. Elements of Risk Assessment and Risk Management (Reproduced from National Research Council, 1983)

Risk assessments are typically implemented using frameworks developed with a particular field or industry in mind. No agreed Australian framework yet exists specifically for intensive animal farming. However, most risk assessments employ similar approaches, and models already exist which appear largely applicable to feedlots. Figure 0-1 shows a scheme developed in the USA in the 1980s with a view to the better management of the risks arising from exposure to toxic chemicals in solid and liquid waste (National Research Council, 1983). This system has since been adapted globally to a diverse range of industries. The Codex Alimentarius (Codex Alimentarius Commission, 1999, WHO and FAO, 2011) used by the food industry internationally, including the livestock industry, is in part based on these same principles. Risk assessment has been central to ensuring safe meat production since the adaption of risk assessment/Hazard Analysis and Critical

Control Points (HACCP) principles developed originally for the NASA space program (Hulebak and Schlosser, 2002):

- Hazard analysis;
- Control points;
- Critical limits;
- Monitoring;
- Management actions;
- Validation/verification;
- Record keeping.

Other local examples of risk assessment and management documents include AS/NZS 4360(Standards Australia/Standards New Zealand, 1999), the Australian Drinking Water Guidelines(NH&MRC NRMMC, 2004) and NHMRC Guidelines for managing risks in recreational waters (NH&MRC, 2008) and Health Impact and Health Risk Assessment Guidelines (Department of Health and Aging, 2002, Department of Health and Aging, 2001). All of these have been developed in part with chemical and pathogen risk management in mind.

Figure 0-2 shows the assessment framework proposed for Feedlots reflecting these pre-existing risk frameworks. The FLOT project structure has been designed to address each of the implied information needs and this report uses these assessment components as its major section headings.

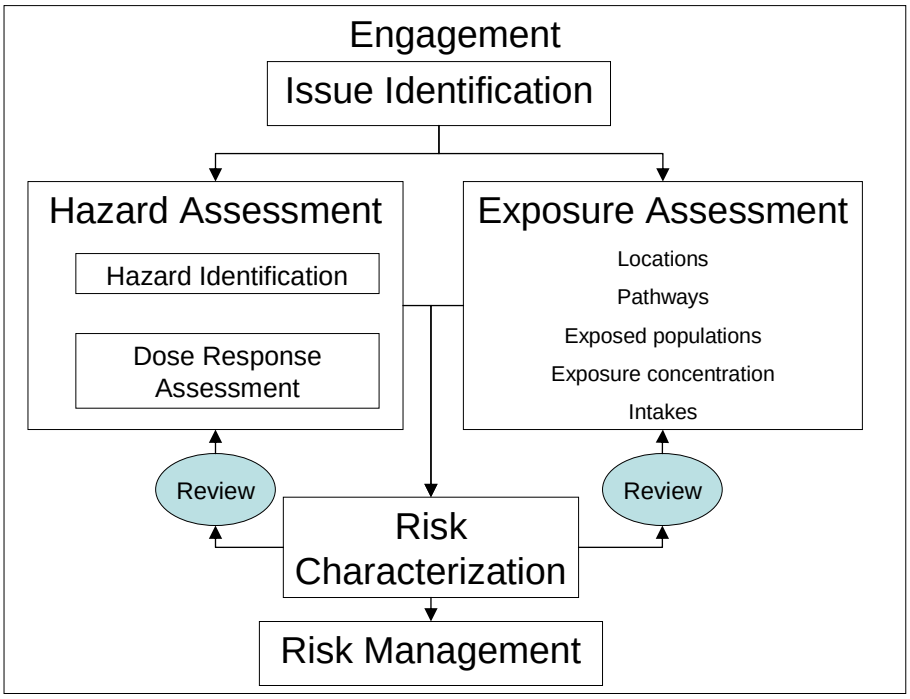


Figure 0-2. Summary of Recommended Health Risk Assessment Process

I.2 Document Structure

Consistent with risk assessment practice and this framework about this report is divided as follows:

- Chapter I Background
- Chapter II. Engagement
- Chapter III Issue Identification
- Chapter IV. Hazard Assessment (comprising)
    - Section IV.1. Hazard Identification
    - Section IV.2. Dose Response Assessment
- Chapter V. Exposure Assessment (comprising)
    - Section V.1. Exposure Locations

    - Section V.2. Exposed Populations
    - Section V.3. Exposure Pathways
    - Section V.4. Exposure Concentrations
    - Section V.5. Contaminant Intake
    - Section V.6. Uncertainties
- Chapter VI Risk Characterization
- Chapter VII. Risk Management Recommendations
- Chapter VIII. References
- Chapter IX. Appendices

The primary risk assessment text, figures and tables (Chapters I to VIII) has been designed as a stand-alone document with detailed information being compiled in stand-alone Appendices. The latter include interim results and papers and to a degree there is redundancy in the information provided. Text and Tables in the primary risk assessment have been at times adapted or updated from the originals in the Appendices for clear communication.

II Engagement

WRC/FSA:

- consulted repeatedly with MLA and presented interim reports;
- consulted with industry (e.g. FSA's Feedlot survey);
- published a range of peer reviewed papers (e.g. Klein et al., 2011, Klein et al., 2010b, Klein et al., 2010a, Khan et al., 2008) during the course of the project (Peer review was seen as providing verification of the acceptability of the methodologies used).
- consulted with US feedlot experts in Texas, Kansas and Iowa and inspected current research activities;
- jointly visited *ca*10 feedlots to define the general attributes of Feedlots for its attention;
- selected 5 feedlots located in 3 different states to act as models and ensure data collected would generate representative contaminant patterns across a large sweep of Australia;
- consulted MLA on management recommendation development with a view to further industry consultation on this document.

FSA provided ongoing guidance to WRC on feedlot operations and procedures and facilitated on-site experiments and sample collection.

MLA provided information on an earlier risk assessment proposal by Alliance Consulting and Management (FLOT 216). As a result of the engagement process 4 documents were developed.

II.1 General End User Analysis

The first major activity, Appendix 1 End-user Analysis, surveyed waste management practices of concern/interest to the feedlot industry. It identified a need to document management practices into a usable guideline form, and facilitate the dissemination and acceptance of the guidelines among stakeholders of the Australian feedlot industry.

It was found that Australia has some 866 beef feedlots with a combined pen capacity of about 1,190,000 head which produce about 527,000 $t.a^{-1}$ of stockpiled manure. Most large feedlots store or compost manure for less than 12 months before spreading. The number of feedlots composting manure has risen from twelve in 2006 to eighteen in 2010. Of the feedlots that compost, almost half add substrates other than manure to the windrow or pile. Four feedlots record windrow temperatures. Six feedlots add freshwater or effluent to the manure. The range in practices reflects developing composting skills. Twenty-seven of the feedlots surveyed screened manure once as usual practice, usually prior to spreading or on-selling. On and off-site manure spreading are common. Twenty-five of the feedlots surveyed undertake on-site spreading. Spreading rates range from less than 5 $t.ha^{-1}$ to >30 $t.ha^{-1}$. The majority spread at a rate of >5-10 $t.ha^{-1}$, although >20-30 $t.ha^{-1}$ is the next most common category. Manure that is spread on-site is mostly used to grow hay or silage crops or grain crops.

Manure exported off-site is used to grow grain, cotton / sugar cane; horticultural crops, hay / silage; pastures and by nurseries / landscapers. Most feedlots irrigate effluent, with about half of the respondents using spray irrigation. Hay / silage crops are the most common land use, although grain crops are also significant. Composting is the most common method for carcass disposal, followed by burial. Most operations which are composting, use windrows rather than piles.

II.2 Existing Waste Management Practice Examples

Appendix 1 was supplemented by Appendix 2 Survey of Existing Waste Management Practices at Model Feedlots. This report documents illustrative waste management practices at a higher level of detail. It was also designed to document the specific management practices at the 5 Feedlots whose manure streams were proposed for study in depth, provide a context for interpreting the full report findings and illustrate waste management approaches of lot feeders. The selected feedlots included operations which:

1. Simply aged harvested manure in windrows (n = 3);
2. Composted the harvested manure without carbon amendment (n = 1);
3. Composted harvested manure with amendment (n = 1).

Increasing use of composting was judged likely in the future and this was taken into consideration during the experimental design phase.

II.3 Composting of Manure

Four manure composting subcontractors were identified (Appendix 3 Composters Case Study). FSA undertook a telephone survey of manure composting contractors about the practices and economic of the services they provide. Key findings included the following:

1. Windrow turners are generally used.
2. The size of compost windrows varies from 1.5-1.8 m high to 2.5 m.
3. Each contractor produces multiple types of composts.
4. At a minimum, all contractors achieve windrow heating to 55°C for three consecutive days prior to three turns.
5. All contractors monitor temperature and moisture content and add moisture as needed throughout the process. Recycled water is used to a degree which could impact quality.
6. Benefits of composting are: consistency of product, friability, easier to handle; does not contain physical contaminants (rock / concrete); is stabilised with respect to nutrients, pathogens and weed seeds; and has reportedly better levels of beneficial fungi and bacteria.
7. It is estimated that composting produces dry matter losses of 30-66% of initial bulk.
8. Typically the composting organisations aimed for a final moisture content in the compost of about 30%, although one aims for 40-55% depending on the application proposed.
9. Nutrient content variation is somewhat unclear.
10. Composters pay feedlots less than $10 plus GST for every tonne of compost they produce.
11. Most is sold in bulk.
12. Bulk sale price is in the region of $25 to $80 per tonne though for small premium product in 20 kg bags the price exceeds $10 per 20 kg wholesale. Other costs reported include ~ $4 km^{-1} for transport and $12 t^{-1} for spreading.
13. All contractors have some type of biological or organic certification, although this can't always be applied to feedlot manure compost.

II.4 Cooperation with Feedlot Operators

With the user analysis and literature review (Appendix 5) in mind an experimental plan was developed and provided to lot feeders who agreed to participate in the experimental survey (Appendix 4 Experimental Project Plan Summary for Feedlot Co-Operators).

It was agreed to not identify cooperating Feedlots in this report. This did not impact at all on this report as broad patterns of contamination proved to be comparable across the feedlots except where there were site-specific differences (e.g. differences in occurrence of parasiticides reflecting different feedlot selection, less microbial contamination in end product which had been composted with the intent in part of reducing pathogen numbers in this material).

III Issue Identification

WRC/FSA:

- inspected operational feedlots in NSW, Queensland and Victoria to identify where risks could occur and designed the survey and selected sample types so as to cover the main contaminated materials, geographical spread and climate variation (Engagement Appendices 1 & 2);
- undertook a literature survey of feedlot risks and contaminants (Appendix 5 Review of Contaminants in Feedlot Wastes);
- undertook a literature review of carcass composting (Appendix 6 Carcass Composting Review);
- undertook a Preliminary Exposure Pathway (Risk) Assessment to identify which pathways were most significant and which human populations were likely to be most exposed based on first principles and hence should be the focus of research and assessment.
  (Note that one outcome was the recognition that despite its high profile, the risk of contaminants in run-off had by in large been contained by current storm-water management basins and aerosols were the main concern) (Appendix 7 Preliminary Assessment of Risk and Refinement of FLOT.333 Project Plan);
- surveyed contaminant levels in feedlot wastes to determine which of the concerns identified in the literature were applicable in Australia (various Appendices – see below);
- undertook *in situ* and *in vitro* measurements of rates of inactivation/decomposition/disappearance of different contaminants to obtain quantitative data on disappearance rates with a view to informing management practices and as a check on patterns of contamination observed in the manure survey (various Appendices – see below).

III.1 Literature review

This literature review (Appendix 5) was designed to be the first stage in 'Hazard Identification' and the 'Dose Response' assessment stages of 'Hazard Assessment'. Table 0-2 summarises the knowledge gaps provisionally identified via the review. Most pertained to Hazard Identification. Also important were gaps in knowledge of the aerosols/dusts and the transport and dissemination of all contaminants.

Table 0-2: Summary of knowledge gaps remaining following the present review

Contaminant/ issue	Knowledge gap
Campylobacter jejuni/coli	Concentrations in bovine waste in Australia Persistence in bovine waste and soil under local conditions Human and/or animal outbreaks of disease from animal waste products
Shiga toxin-producing *E. coli* (STEC)	Persistence particularly of non-0157:H7 in bovine waste and soil
Salmonella spp.	Concentrations in bovine waste in Australia
Coxiella burnetii	Concentrations in bovine waste in Australia Persistence in bovine waste, soil and dust
Leptospira spp.	Persistence in bovine waste and soil
Listeria spp.	Persistence in bovine waste and soil under local conditions
Yersinia	Concentrations in bovine waste in Australia Human and/or animal outbreaks of disease from animal waste products Infectious dose Persistence in bovine waste and soil
Clostridium spp.	Concentrations in bovine waste in Australia Persistence in bovine waste and soil
Mycobacterium avium subsp. *paratuberculosis*	Concentrations in bovine waste in Australia
Mycotoxins	Concentrations in bovine waste in Australia Human and/or animal poisoning from animal waste products Degradation processes in bovine waste and soil

Contaminant/ issue	Knowledge gap
Cryptosporidium spp.	Concentrations in bovine waste in Australia
Giardia spp.	Concentrations in bovine waste in Australia Persistence in bovine waste and soil
Fungi / mycotoxins	Evidence of mycotoxin-producing organisms growing in feedlot grains Assessment of likelihood of mycotoxin production under various management practices
Viruses	Concentrations and types in bovine waste in Australia Persistence in bovine waste and soil
Aerosols and dust	Concentrations Travel distance
Helminths	Not a significant gap
Cyanobacteria	Not a significant gap
Prions	Not a significant gap
'Shipping fever'	Does it predispose cattle to other infections?
Hormones	Concentrations in feedlot waste in Australia Persistence in bovine waste and soil
Antibiotics	Concentrations in feedlot waste in Australia Persistence in bovine waste and soil Effect on soil ecology Nature of risk
Pesticides	Concentrations in feedlot waste in Australia Persistence in bovine waste and soil Effect on arthropod ecology
Heavy metals	Not considered significant
All	Transport and dissemination

III.2 Carcass Composting

Mortality disposal by composting (Appendix 6 Carcass Composting Review) is becoming increasingly popular in Australian beef feedlots. It is generally the favoured mortalities disposal method with those responsible for administering environmental regulation as it can avoid the potential impacts from other methods. For example, burial may pose a threat to groundwater and surface water quality; while burning can cause air pollution and odour nuisance. From a health perspective, it is a good option if done well as material within the pile reaches temperatures that are sufficient to kill many types of pathogens. It is popular with industry because it is practical, low cost, can provide for good biosecurity and produces a valuable soil amendment. For instance, there is no need to excavate pits for burial; or to transport mortalities off-farm for rendering. Quick removal of mortalities from the vicinity of live cattle and managed to minimise disease transfer risks.

Feedlots largely follow the mortality composting practices adopted from the poultry and pork industries that have used this method for many years although there is also some on-site experimentation. This report represents a review of scientific literature on cattle mortality composting with a focus on practices that will achieve the high temperatures needed to reduce pathogen survival and transfer.

III.3 Exposure Pathway Scoping

Though feedlots are relatively simple systems compared to natural environments and their designs are standardized it was clear from this initial scoping that we needed to prioritize the contaminants to be considered and the pathways by which different populations might be exposed. So bearing in mind waste management practices (Appendices 1 & 2) and contaminants (Appendix 5) we undertook a Preliminary Exposure Pathway Assessment (PEPA) based on qualitative risk assessment approaches (e.g. as used with water Nadebaum et al., 2004). The process is outlined below in Sections V.1. Exposure Locations, V.2 Exposed Populations and V.3. Exposure Pathways and detailed in Appendix 7.

From the PEPA, exposure to contaminant-laden aerosols particularly dry dusts was identified as a primary issue for consideration. For risk assessment the main data needs were identified as being:

- Inhalation rates by populations of dust – exposure time X inhalation volume (standard rates are available in the literature);
- Concentration of contaminants in aerosolisable material (pad manure, aged manure, compost - planned as part of the Feedlot contaminant survey);
- Dust emission rates and persistence of the aerosol source material in the exposure zone under different scenarios e.g. disturbance by machinery or cattle, gardening using compost.

With the last of these data needs in mind an Aerosol Measurement Campaign Plan (Appendix 8) was developed to quantify cattle feedlot dust aersolization during dry conditions. Specialists at Queensland University of Technology(QUT) with expertise in particle size monitoring and analysis were subcontracted to undertake the work. WRC/FSA worked with QUT to identify emission scenarios of concern. These were seen as occurring in two basic forms:

- Long term emissions from the feedlot area concentrations e.g. over several days;
- Short term emissions from specific disturbance scenarios.

IV Hazard Assessment

IV.1 Hazard Identification

IV.1.1 Priority Contaminants

From Appendix 5 Review of Contaminants in Feedlot Wastes and Appendix 7 Preliminary Assessment of Risk and Refinement of FLOT.333 Project Plan the hazards of greatest potential concern were identified as zoonotic pathogens, endocrine disrupting compounds and parasiticides. The final list of contaminants proposed for monitoring, and the actual list surveyed, are shown in Table 0-3.

All pathogens searched for were detected though in varying abundance. All steroid groups were detected. Deltamethrin, cypermethrin, flumethrin and eprinomectin were not detected in all samples while abamectin and ivermectin were detected in most of the samples.

Table 0-3. Contaminants and other Parameters Proposed or Actually Measured

Contaminant class	Proposed Model Contaminant/ Parameter[a]	COPC[b]?	Comments/Examples
Pathogen	***E. coli* O157/EHEC**	Yes	
	Cryptosporidium		
	Campylobacter		
	Giardia		
	Aspergillus		
	Salmonella		
	Leptospira* or *Mycobacterium		
	Coxiella burnetii		
	Listeria monocytogenes		
	Yersinia pseudoturberculosis		
Microbial Indicator	***C. perfringens***	No	Measurement of these would be aimed at estimating process rates in a cost efficient manner.
	enterococci		
	E. coli		
Ectoparasiticides	Aminidines	Yes	Amitraz
	Benzylphenyl urea		Fluazuron
	Macrocyclic lactones		abamectin, doramectin, ivermectin and eprinomectin
	Synthetic pyrethroids		deltamethrin, cypermethrin, flumethrin
Steroidal hormones	**Estrogenic hormones**	Yes	Estradiol, estrone
	Androgenic hormones		Trenbolone, Testosterone, Testosterone metabolites
	Progestinal hormones		Progesterone
Antibiotics	Virginiamycin	Yes	
	Tylosin		
	Oxytetracycline		
Problematic Contaminants	***Antibiotic Resistance Genes***	Yes	The risk assessment was expected to provide insights into these problems and how they might be controlled rather than develop specific risk estimates or rating.
	Weed seeds	No	
	Lipopolysaccharrides	Yes	
	Mycotoxins	Yes	
	Organic Dust Toxicity Syndrome (ODTS) agents	?	
Physico-chemical parameters	**Temperature profiles (air, stockpiles)**	No	Particle size profiles of manure and compost would be measured in the laboratory to assess their potential for dispersion especially as aerosols. Spot measurements
	Humidity		
	Solar radiation		

Contaminant class	Proposed Model Contaminant/ Parameter[a]	COPC[b]?	Comments/Examples
	Water content		of actual emission and dispersion rates would be undertaken in the field at a northern and a southern feedlot.
	Total mass of material		
	Particle size distributions		
	Wind speed and direction		
	Regional climatological parameters (rainfall, sunlight, temperature, humidity)		
Other Measurements	**Modelling of particle transport**	No	
	(Modelled) Risk estimates		@Risk (MS Excel)
	Manure management characteristics		See user

a. Parameters measured or estimated in the surveys and subsequent work are shown in **bold**.
b. Contaminant of Potential Concern.

IV.1.2 Contaminant Survey

IV.1.2.1 Principle findings

Experimental work commenced in mid-2008. Method development and interim progress is detailed in Appendix 9 Interim Results Report. All the pathogens tested for were detected in at least one manure sample. The most frequently found pathogen groups were pathogenic *Escherichia coli* (EHEC or EPEC based on levels of the intimin gene), *Listeria monocytogenes*, and *Campylobacter jejuni*, followed by the protozoan pathogens *Giardia* and *Cryptosporidium*. Longitudinal comparisons show that manure aging and management was associated with greatly reduced pathogen load compared to the numbers present in fresh faeces, but obtaining elevated temperatures throughout manure profiles in the absence of deliberate composting was unlikely.

The two dominant androgens detected in most feedlots were etiocholanolone and testosterone propionate. Etiocholanolone is a metabolite of testosterone and was ubiquitous in all sample types from four of five feedlots. The estrogenic steroidal hormones 17α-estradiol and/or 17β-estradiol were generally detected in all feedlots. While the limitations of the data made it difficult to assess trends, it appeared that concentrations of estradiol (mass per dry weight) tended to decrease as manure aged. This observation was consistent with previous investigations indicating that these estrogenic steroidal hormones are gradually oxidised to form the metabolite, estrone. Different residuals of macrocyclic lactones (doramectin, ivermectin, eprinomectin and abamectin) were detected at different feedlots but no striking trends were seen regarding their degradation and ultimate fate.

IV.1.2.2 Method Development

IV.1.2.2.1 Pathogens

A key challenge for pathogens was how to cost effectively survey a large number of different species given the specialized and diverse nature of assays used historically. The decision was made to investigate the utility of quantitative real-time PCR (qPCR) for surveying the different microbial constituents of different faecal wastes (Appendix 10 Monitoring Bacterial Indicators and Pathogens in Cattle Feedlot Waste by Real-Time PCR). To validate the approach the abundances of *Escherichia coli* and enterococci were estimated in five cattle feedlot waste types from five localities. Bacteria were quantified concurrently using two culture methods and compared to the number of genome copies detected by qPCR targeted at *E. coli* and *Enterococcus faecalis*. Bacterial numbers detected in the different wastes (fresh faeces, pen manure, aged manure, composted manure, carcass manure compost) ranged from 10^7 to 10^2 g^{-1} (dry weight). Both indicator groups were detected by qPCR with a comparable sensitivity to culture methods across

this range. QPCR measurements of *E. coli* and *E. faecalis* correlated well with MPN and spread plate data. As a second comparison we inoculated green fluorescent protein (GFP) labelled reference bacteria into manure samples. GFP labelled *E. coli* and *Listeria monocytogenes* were detected by qPCR in concentrations corresponding to between 18% and 71% of the initial bacterial numbers, compared to only 2.5% to 16% by plating. Our results supported our selection of qPCR as a fast, accurate and reliable system for surveying pathogens in cattle waste.

IV.1.2.2.2 Ectoparasiticides

In the case of ectoparasiticides a rapid high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analytical method was developed for the simultaneous analysis of 3 synthetic pyrethroids (deltamethrin, cypermethrin, flumethrin) and 4 macrocyclic lactones (abamectin, doramectin, ivermectin and eprinomectin) in aqueous matrices and animal feedlot waste. This method is unique in its inclusion of all 7 of these synthetic pyrethroids and macrocyclic lactones and of particular value due to its very short chromatographic run time of 16 minutes. Method recoveries of analytes in various matrices were from 48 to 110%. Method detection levels (MDLs) were determined to describe analyte concentrations sufficient to provide a signal with 99% certainty of detection. The established MDLs for all analytes were 0.6-10 $ng.L^{-1}$ (equal to 1.2– 20 $\mu g.kg^{-1}$ freeze dried feedlot waste) in a variety of matrices. The method was applied to analyse feedlot samples taken after various stages of processing from an Australian operational beef cattle feedlot. Further details are provided in Appendix 11 Ectoparasiticides in Australian Beef Cattle Feedlot Wastes.

IV.1.2.2.3 Steroidal Hormones

Gas chromatography-mass spectrometry (GC–MS) has been a preferred technique for determination of steroidal hormones as it is generally able to achieve improved detection limits in more complex matrices (Regan et al., 2002, Mol et al., 2000, Xiao et al., 2001, Ding and Chiang, 2003, Song et al., 2003, Liu et al., 2004, Shareef et al., 2004, Meunier-Solère et al., 2005, Budzinski et al., 2006, Kootstra et al., 2007, Magnisali et al., 2008). Further improvements in sensitivity have been achieved by GC coupled with tandem mass spectrometry (GC-MS/MS). A number of GC-MS/MS methods have been developed for the analysis of estrogenic steroidal hormones in biological and environmental samples (Zhang and Zuo, 2005, Kelly, 2000, Jeannot et al., 2002, Quintana et al., 2004, Stanford and Weinberg, 2007). A few GC-MS/MS methods have been developed for the analysis of a wider range of steroidal hormones including a few androgens and progesterone (Van Vyncht et al., 1994, Kolodziej et al., 2003). However, no methods are currently available for the rapid simultaneous determination of all 6 estrogens, 7 androgens and progesterone. Furthermore, the previously published GC-MS/MS methods that have included simultaneous analysis of some estrogenic and androgenic hormones have not incorporated isotope dilution for accurate quantitation accounting for extraction losses and potential matrix effects (Van Vyncht et al., 1994, Kolodziej et al., 2003).

In order to overcome the above limitations, we developed a simple, rapid, reliable and sensitive analytical method for the simultaneous determination of the most common 6 steroidal estrogens, 7 androgens and progesterone in environmental matrices and animal feedlot wastes (Appendix 12 Estrogens, Androgens and Progesterone in Solid Waste). Solid wastes from feedlot operations are extracted by ultrasonication followed by solid phase extraction (SPE), while water matrices are extracted by SPE. The method incorporates GC-MS/MS analysis using isotope dilution for accurate quantitation. With the exception of the synthetic androgenic hormone Trenbolone, which requires an additional derivatisation step, the analytes can be monitored in a single GC-MS/MS run with a run time of 15 minutes.

IV.1.2.3 Pathogen Content of Manures

The presence of the eight bacterial contaminants and two pathogenic Protozoa were analysed by real-time PCR (qPCR) in cattle manure from the five beef cattle feedlots during summer and winter (3 enclosures). Most samples tested positive for one or more pathogens of concern in numbers range over five orders of magnitude. The most abundant pathogens were enterohaemorrhagic /

enteropathogenic *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Giardia* spp. and *Cryptosporidium* spp., followed by the less frequent pathogens *Yersinia pseudoturberculosis*, *Salmonella enterica*, *Coxiella burnetii*, pathogenic *Leptospira*, and *Mycobacterium avium paratuberculosis*. Bacterial indicator levels were also quantified. Table 0-4 shows grand summary statistics for each pathogen and indicator and waste material. Table 0-5 summarises the frequency with which different organisms were detected.

Both indicators and pathogens appeared to be more rapidly inactivated during summer. The efficiency of sample preparation and the ability to quantitate pathogens by qPCR was examined using a range of type organisms reinoculated into different manures. Recoveries within one order of magnitude of the levels inoculated were observed, confirming qPCR as an analytical tool suited to quantitatively surveying microbial contamination in hard-to-analyse cattle feedlot manure streams. Further details can be found in Appendix 13 Pathogens and Indicators in Cattle Feedlot Manure –Primary Survey Results.

By comparing pathogen numbers and occurrence frequency with indicator numbers it was possible to assess the reliability of conventional indicator tests as measures of the presence or otherwise of pathogens (Appendix 14 Indicator Monitoring Reliability). It was concluded that:

1. Indicator disappearance does reflect pathogen reduction but the match is not perfect.
2. In the absence of enterococci assayed by qPCR other pathogens should be undetectable as well.
3. qPCR appears relatively more sensitive to indicator presence than cultural methods even though the sample sizes were smaller.
4. Direct pathogen monitoring is feasible and storage of DNA extracts could allow future monitoring to be based on qPCR as well as conventional indicator testing.

Further Supplementary Survey Data on Pathogens in Feedlot Wastes is documented in Appendix 15 which may be of use/interest:

1. A breakdown of sample analysis numbers between winter and summer, each material and southern and northern feedlots.
2. Variation in pathogen detection between these different sample types.
3. Other statistics describing the numbers of pathogens and indicators observed which may be preferred for some purposes.
4. Further details of pathogen and indicator probability density function estimation (some data in Appendix 13 are reproduced).
5. A summary description of the final probability density functions used in calculating pathogen risk.

Table 0-4. Log_{10} mean and standard deviation coefficients for microbial populations in cattle feedlot wastes.

Analyte	Method	Arithmetic mean of log_{10} numbers g^{-1} ± 1 SD					
		Fresh faeces	Pen manure	Harvested manure	Aged manure	Compost manure	Carcass compost
Total coliforms	**MPN**	7.4±0.28	6.1±1.1	3.2±1.9	2.7±1.28	2.3±1.2	*3.6*
E .coli	**MPN**	7.4±0.33	5.2±1.3	2.5±1.6	1.6±0.54	*1.0*	1.1±0.32
	qPCR	6.8±0.69	5.1±0.98	3.5±1.5	2.5±0.56	*2.8*	ND
Faecal enterococci	**MPN**	5.8±0.73	5.2±0.88	3.1±1.5	1.7±0.69	2.4±1.6	2.0±1.2
E. faecalis	**qPCR**	6.2±0.87	6.0±0.84	4.9±0.95	3.9±0.50	3.6±0.38	4.6±1.00
C. perfringens	**qPCR**	4.5±0.75	3.8±0.87	3.8±0.98	3.7±0.69	ND	4.3±0.80
pathogenic *E. coli* [a]	**qPCR**	5.1±1.34	3.8±1.9	2.6±0.85	2.5±0.59	*2.6*	2.6±0.78
C. jejuni	**qPCR**	5.1±0.94	3.3±0.66	ND	*2.9*	ND	ND
C. parvum	**qPCR**	3.7±0.94	3.2±0.71	*3.0*	3.1±0.67	ND	ND
G. lamblia	**qPCR**	4.6±1.65	ND	*3.0*	3.5±0.98	ND	3.9±0.97
L. monocytogenes	**qPCR**	3.7±0.53	3.2±0.53	3.0±0.38	3.2±0.61	ND	3.4±0.64
S. enterica	**qPCR**	*3.4*	ND	ND	ND	ND	ND
C. burnetii	**qPCR**	*3.4*	ND	*2.9*	ND	ND	ND
Leptospira spp.	**qPCR**	ND	*3.0*	ND	ND	ND	ND
M. paratuberculosis	**qPCR**	ND	ND	*3.0*	ND	ND	ND
Y. pseudotuberculosis	**qPCR**	*3.4*	*3.0*	*2.9*	*2.9*	ND	ND

a. positive for virulence gene eaeA
b. Quantification of manure samples was performed by qPCR or culture assay as described in Materials and Methods.
c. The detection limits for pathogens by qPCR were *ca* 3.0 log_{10} units for fresh waste and *ca* 2.5 for aged material and *ca* 1.0 for culture based assays.
d. ND represents analysis under the detection limit.
e. Numbers without SD value correspond to those where the pathogen was detected in less than three samples

Table 0-5. Frequency of detection of indicators and pathogens in feedlot wastes.

Analyte	Method	% Detection (number of analyses)					
		Fresh faeces	Pen manure	Harvested manure	Aged manure	Composted manure	Carcass compost
Total coliforms	**MPN**	100 (17)	100 (16)	79 (14)	79 (14)	67 (3)	100 (3)
E. coli	**MPN**	100 (32)	100 (30)	68 (28)	57 (23)	17 (6)	43 (7)
	qPCR	100 (32)	94 (31)	56 (25)	5 (20)	33 (6)	**0 (7)**
Faecal enterococci	**MPN**	100 (28)	100 (32)	79 (28)	61 (23)	67 (6)	57 (7)
E. faecalis	**qPCR**	100 (32)	100 (32)	100 (25)	95 (20)	100 (6)	100 (7)
C. perfringens	**qPCR**	82 (17)	65 (17)	64 (11)	55 (11)	**0 (3)**	67 (3)
pathogenic *E. coli* [a]	**qPCR**	81 (32)	69 (32)	32 (25)	20 (20)	17 (6)	14 (7)
C. jejuni	**qPCR**	94 (32)	38 (32)	**0 (25)**	5 (20)	**0 (6)**	**0(7)**
C. parvum	**qPCR**	13 (32)	16 (32)	8 (25)	15 (20)	**0 (6)**	**0 (7)**
G. lamblia	**qPCR**	34 (32)	**0 (32)**	8 (25)	30 (20)	**0 (6)**	43 (7)
L. monocytogenes	**qPCR**	31 (32)	34 (32)	16 (25)	35 (20)	**0 (6)**	43 (7)
S. enterica	**qPCR**	6 (32)	**0 (32)**	**0 (25)**	**0 (20)**	**0 (6)**	**0 (7)**
C. burnetii	**qPCR**	3 (32)	**0 (32)**	4 (25)	**0 (20)**	**0 (6)**	**0 (7)**
Leptospira spp.	**qPCR**	**0 (32)**	3 (32)	**0 (25)**	**0 (20)**	**0 (6)**	**0 (7)**
M. paratuberculosis	**qPCR**	**0 (32)**	**0 (32)**	8 (25)	**0 (20)**	**0 (6)**	**0 (7)**
Y. pseudotuberculosis	**qPCR**	3 (32)	6 (32)	4 (25)	5 (20)	**0(6)**	**0 (7)**

a. positive for virulence gene eaeA
b. Quantification of manure samples was performed by qPCR or culture assay as described in Materials and Methods. Assays under the detection limit are shown in bold face.

IV.1.2.4 Chemical Content of Manures

Full results of the ectoparasiticide analyses are detailed in Appendix 11 Ectoparasiticides in Australian Beef Cattle Feedlot Wastes. Deltamethrin, cypermethrin, flumethrin and eprinomectin were not detected in any samples while abamectin and ivermectin were detected in most of the samples with concentration ranging from 0.6 to 1.3 $\mu g.kg^{-1}$ freeze dried feedlot waste and 5 to 34 $\mu g.kg^{-1}$ freeze dried feedlot waste respectively. Doramectin was only detected in aged manure with concentration of 2.4 $\mu g.kg^{-1}$ freeze dried feedlot waste.

Due to the lack of any clear trends, worst-case concentrations were estimated for use in the risk assessment calculations. This involved identifying an upper-limit concentration for each hormone, based on the monitoring of all types waste material samples across all five feedlots. This upper limit value was set at, or slightly above, the highest concentration reported in any sample. For parasiticides which were not detected in any sample, the upper-limit value was set at the analytical detection limit. The final upper-limit concentrations are shown in Table 0-6.

Table 0-6. Upper-limit concentration values use for steroidal hormones in risk analysis calculations.

Analyte	Concentration - Upper Limit ($\mu g.kg^{-1}$)
Abamectin	20
Ivermectin	40
Eprinomectin	5
Doramectin	40

The results of the steroidal hormone analyses are detailed in Appendix 12 Estrogens, Androgens and Progesterone in Solid Waste. There was no clear trend with concentrations of hormones in the different samples.

As with the ectoparasiticides, due to the lack of clear trends, worst-case concentrations of steroidals were estimated for use in the risk assessment calculations. This involved identifying an upper-limit concentration for each hormone, based on the monitoring of all types waste material samples across all five feedlots. This upper limit value was set at, or slightly above, the highest concentration reported in any sample. For hormones which were not detected in any sample, the upper-limit value was set at the analytical detection limit. The final upper-limit concentrations are shown in Table 0-7.

Table 0-7. Upper-limit concentration values use for steroidal hormones in risk analysis calculations.

Analyte	Concentration - Upper Limit ($\mu g.kg^{-1}$)
Androsterone	120
Etiocholanolone	1300
Dihydrotestosterone	5
17α-Estradiol	80
Testosterone propionate	1200
Estrone	130
Trenbolone	50
Androstenedione	400
17 β-Estradiol	25

Analyte	Concentration - Upper Limit (µg.kg^{-1})
Testosterone	50
17 α-Ethinylestradiol	80
Progesterone	400
Estriol	1

IV.1.3 Uncertainties

A number of further analytes/parameters were identified as being of potential interest but were not measured directly, assessed indirectly and/or omitted from consideration in the formalized risk assessment.

Aspergillus was excluded as it is more associated with grain and silage which were seen as secondary concerns compared to the manure. The synthetic pyrethroids and macrocyclic lactones highlighted in Table 0-3 were chosen as models for ectoparasiticides as these were widely used (see User Survey). Others may be encountered at other feedlots.

Samples were not assayed for antibiotics since the primary concern was not seen as the antibiotics *per se* but rather the occurrence and fate of antibiotic resistance genes which might eventually be transferred to human pathogens. The potential for the persistence of antibiotic resistance genes was explored indirectly by measuring the disappearance of bacterial genes generally and model Green Fluorescent Protein genes especially. The potential for weed seed persistence was explored indirectly by characterizing windrow temperature gradients.

Endotoxins, mycotoxins and on Organic Dust Toxicity Syndrome (ODTS) causing agents were not directly measured. Endotoxin was not measured directly in this project because literature data was probably sufficient if risk assessment were seen as needed. The range of types and structures of mycotoxins in the literature was judged too diverse for sampled to be meaningfully assayed with available resources. Little work appears to have been undertaken to determine their concentrations in compost material, but direct measurement would be unlikely to yield useful data for FLOT.333. No analytical work could be undertaken at this stage on ODTS inducing compounds because the etiology and cause of this problem (the Hazard) is not defined and hence there was no specific contaminant to enumerate.

As with all microbial and chemical analyses, assay detection limits were constrained by current technology (e.g. only small qPCR samples can be analysed). However, all contaminants were detected in at least one sample and the assays did provide estimates of the upper numbers/concentrations of all contaminants which could be used in estimating the upper limits of risk – as with the ectoparasiticides and steroidal hormones.

A final concern of note, which was resolved, was the initial detection of very high numbers of ‘pathogenic Leptospira’. This proved to be a methodological artefact. Initially *Leptospira* spp. were assayed using a qPCR primer/method developed by the Australian reference laboratory in Queensland. Data obtained using this assay suggested virtually all samples contained *Leptospira* at levels up to 10^8.g^{-1} manure. These numbers were inconsistent with the low/sporadic reporting of this disease at feedlots and a lack of previous reports of high levels (in contrast to pathogenic *E. coli* which have been frequently reported by other authors). So the qPCR primers were re-evaluated and alternatives identified (Appendix 16, Pathogenic Leptospira - False Positive Detection, Improvement of Quantification Method and Reanalysis of Feedlot Wastes). The capacity to store extracted DNA allowed samples to be re-analysed with no loss of data. While Leptospira were again detected the levels and frequency were very low.

IV.2 Dose Response Assessment

IV.2.1 Pathogens

Dose response algorithms or their equivalent were identified from the literature (see also Appendix 5). Where possible data for human related benchmarks, studies and algorithms were selected. Where these were unavailable animal response/models were used. Candidate pathogen dose response algorithms are summarized in Appendix 17 Pathogen Dose Response. Most are ingestion based except for *Coxiella* whereas review of the exposure pathway indicated both dust inhalation and ingestion needed to be considered.

In the following instances it was necessary to infer a conservative dose response algorithm based on the assumption that dose response followed an exponential relationship (Haas and Eisenberg, 2001):

1. In the case of *Yersinia pseudotuberculosis* an infectious dose of 10^3 organisms has been reported. Based on tabulated dose response data this appeared to correspond to a 40% probability of infection. These values were used to estimate the exponential infection dose response coefficient on this assumption.
2. Similarly for a conservative estimate for *Mycobacterium* infection reported by Rusin et al. (1997)10^4 organisms (mice). Again we assumed an exponential relationship and estimated the coefficient using MS Solver assuming this level corresponded to a 50% chance of infection.
3. In the case of *Coxiella* the literature reports high infectivity but the precise infectious dose was unclear beyond secondary references to median dose of "<10 organisms" (e.g. Azad, 2007). In the present instance we interpreted this as suggesting a dose of 1 or 10 organisms had a 50 percent chance of being infectious and estimated the coefficients for the corresponding exponential curves.
4. Leptospires are reported to be highly infectious (Silva et al., 2008, Mori and Arimitsu Y, 1974). To obtain an indication of its infection potential we assumed as with *Coxiella* that a dose of *ca* 10 organisms has a *ca* 50 percent chance of being infectious and estimated the coefficient for an exponential curve.
5. In the case of *Cryptosporidium* the Iowa strain dose response was selected on account of the project dealing with bovine *Cryptosporidia.*

IV.2.2 Chemicals

Chemical dose response thresholds were based on Acceptable Daily Intake (ADI) data acquired from two key sources. These were the 'ADI List' maintained by the Department of Health and Aging, Australia (Office of Chemical Safety, 2011), and the Australian Guidelines for Water Recycling (Phase 2) (Natural Resource Management Ministerial Council et al., 2008). For chemicals for which no established ADI could be identified, ADIs were estimated based on relative potency of the chemical to another chemical for which an established ADI was available. Details are provided in Appendix 18 Chemical Dose Response Algorithms.

IV.2.3 Uncertainties

IV.2.3.1 Pathogens

There are many uncertainties associated with the pathogen dose response algorithms which would tend to make risk estimates conservative e.g.:

1. Pathogen viability was unlikely to be 100%.
2. A pathogen may still have an intact genome but be damaged sufficiently to be incapable of reproduction.
3. Subtypes of the same pathogens are well known to vary in their infectivity e.g. *Salmonella, Cryptosporidium* (Oscar, 2004, Teunis et al., 2002). For the most part

we used conservative algorithms (i.e. assumed high infectivity) except where indicated.
4. The algorithms assume generally that one genome corresponds to one infectious unit, however aggregation of pathogen cells is possible(Teunis et al., 2008).
5. Ingested and inhaled dust would be trapped to a degree and expelled through coughing and sneezing.
6. Some relationships were based on animal models.
7. As evident from our need to derive approximate algorithms above the dose response for Leptospires, *Coxiella*, *Yersinia* and Mycobacteria and not well defined yet compared to other pathogens e.g. *Salmonella*.
8. Microbial dose response curves invariably have high uncertainty boundaries (Teunis et al., 2002).

As a result and risk probability estimates were likely to be quite conservative and at best order of magnitude in precision.

IV.2.3.2 Chemicals

Chemical risk assessment was also likely conservative by virtue of the use of upper limit values.

Uncertainties included:
1. Varying applicability of the underlying toxicology data used to develop ADIs.
2. Variable applicability of the relative potency data for chemicals for which no ADI was directly available.

V Exposure Assessment

V.1 Exposure Locations

Potential exposure locations were identified by inspections of feedlots and discussion on manure/waste management. The relative significance of different exposure locations (termed compartments) was determined by the analysis of the number and length of possible exposure pathways (Appendix 7 Preliminary Assessment of Risk and Refinement of FLOT.333 Project Plan). Based on frequency of occurrence, the priority compartment groups for study were:

1. Pen manure (contaminant source);
2. Manure/compost especially at the feedlot;
3. Atmospheric compartments;
4. Skin and clothing of exposed populations.

The other compartments of interest in approximate decreasing order of importance were:

5. Other animals (insects and horses);
6. Machinery handling;
7. Transport long distance; and
8. Soil.

Exposure was likely to occur in the form of aerosols and dust so both were considered.

V.2 Exposed Populations

The populations identified in the PEPA (Appendix 7) as most likely to be exposed were:

1. Internally to Feedlots:
    a. Feedlot workers;
    b. Visitors;
    c. Subcontractors managing manure.
2. External to Feedlots:
    a. Farmers where manure is applied;
    b. Garden and landscape users of manure/compost;
    c. The public comprising:
        i. Motorists passing manure and cattle trucks.
        ii. Neighbours

Exposure scenarios were developed to cover all these populations.

V.3 Exposure Pathways

Exposure pathways were identified from Feedlot observations by scoping how contaminants were likely to be transferred between different conceptual ‘compartments’ (Appendix 7) and sorting material transfer pathways in order of those most likely to lead to exposure. Of the 338 between compartment transfers considered, 60 were identified as being of primary potential concern for pen manure management. Based on this analysis the most important generic between-compartment transfer processes needing to be characterised/researched in terms of total load, and conditions that affect total load were:

1. Aerosolisation of manure and compost dust and its transfer in atmosphere from point of production to downwind sites;
2. Transfer of contaminated dust to clothing and subsequent ingestion;
3. Inhalation and ingestion of dust;
4. Transport (truck) systems off-site;
5. Insect (fly) borne export.

The first four of these were focused on because of the large quantities of material transferred. MLA expressed interest in run-off and this has been included for completeness. Exposure locations, pathways and their apparent significance are summarized in Figure 0-3.

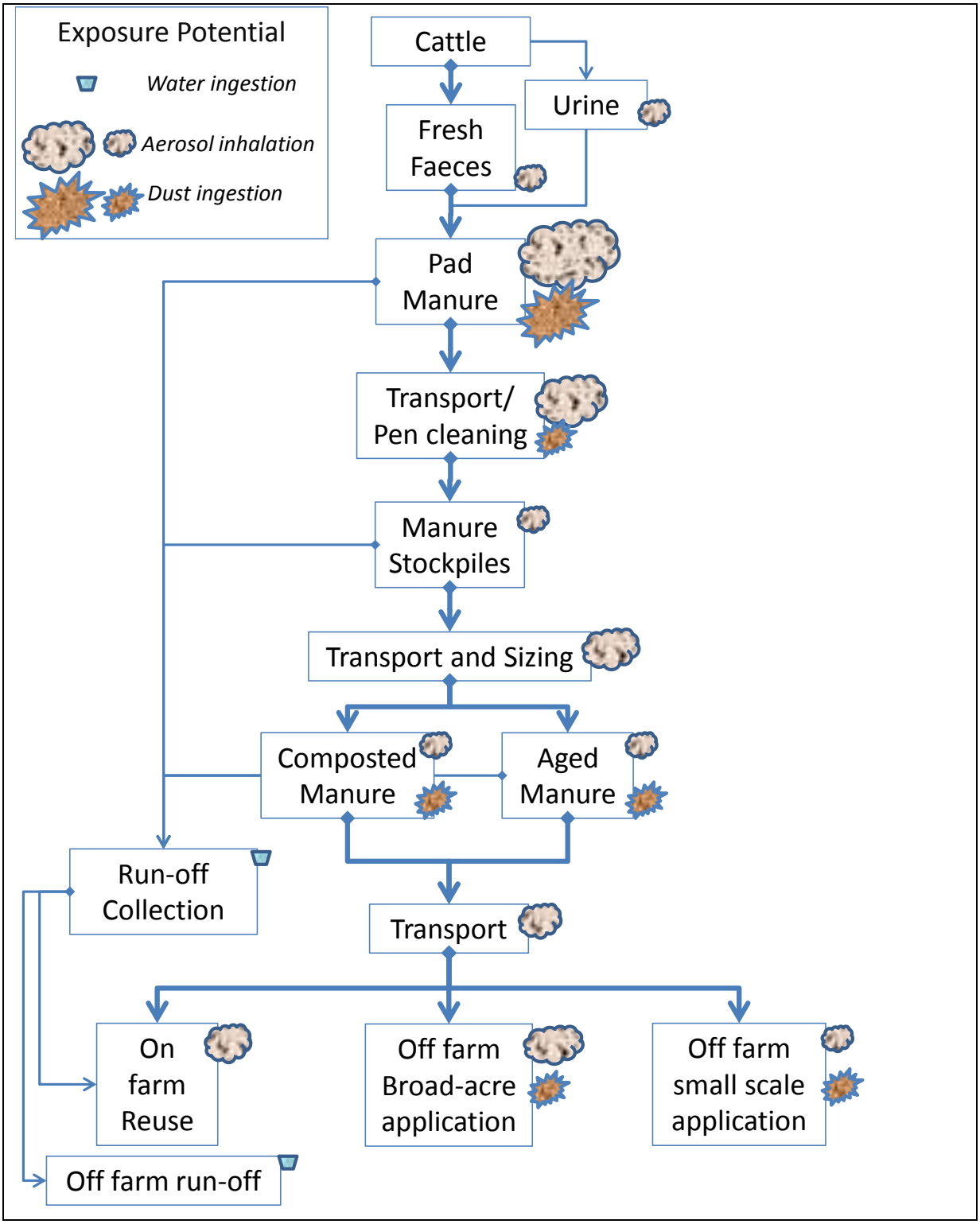


Figure 0-3. Exposure pathways showing locations of manure contaminants where exposure is likely and how they are linked to one another.

a. Relative potential exposure is illustrated by the size of the icons
b. Large arrows indicate the main contaminant transport pathways.

V.4 Exposure Concentrations/Levels

Exposure concentrations/levels were calculated by combining estimates of the contaminant concentrations/levels in feedlot wastes with estimates of the concentrations of manure wastes to which different populations were exposed under different scenarios.

V.4.1 Contaminant Concentrations/Levels in Aerosols and Dusts

The concentrations/levels of contaminants were estimated in the first instance directly through the waste contaminant survey (Section IV.1.2 Contaminant Survey). The utility of this primary analytical data was limited by assay sensitivity. Further it was desired to estimate the level of contaminants would be present after extended composting or aging. Therefore some pathogen risk scenarios assumed a reduction by 3 or 5 decimals (log_{10} units) compared to harvested manure consistent with reductions probably achievable through composting (next section).

V.4.2 Inactivation/Reduction/Decomposition

In order to more completely quantify the impact of manure aging and composting on pathogens a range of experiments were undertaken (Appendix 19 Inactivation Kinetics of Model Microorganisms). The survival of indicators and pathogens in faecal pen manure, stockpiled manure and manure compost was measured using autochthonous indicator bacteria (*Escherichia coli*, *Clostridium perfringens*, enterococci, total coliforms) and pathogens (*Listeria monocytogenes, Campylobacter jejuni*) using culture and/or real-time PCR (qPCR) methods. Additionally, the manures were incubated at 20, 37, 50 and 60°C in microcosms to quantify the persistence of autochthonous microorganisms and selected process performance surrogates (*C. sporogenes,* green fluorescent protein [GFP] labelled *E. coli* and *L. monocytogenes*) given different degrees of composting. QPCR based cell counts indicated that up to four orders of magnitude more target cells were present compared to culturable counts. Corresponding T_{90} estimates were up to 6-fold higher.

The key microcosm experimental data are shown in Table 0-8. They illustrate the clear temperature dependency of pathogen inactivation. Further analysis of the relationship between temperature and inactivation is presented in Appendix 20 Inactivation Rate Kinetics Temperature Dependency. Depending on the model used T_{90}s for vegetative cells based on qPCR assays were in the range 1 to 10 days above 55 ^{o}C in the relatively dry manures used in the inactivation experiments (Figure 0-4). It was concluded after 2 to 3 months of composting most pathogens should have been reduced in numbers by 3 or more log_{10} units consistent with the low numbers of pathogens and indicators in composted manures from the Feedlot#2 and Feedlot#3 Feedlots where composting was undertaken (noting microbial contaminants were not completely absent in the samples analysed).

The persistence of chemicals during aging and composting was also measured. However, no clear trends were observed. Therefore for risk characterizations it was decided to use the upper limit concentration values in all scenario calculations (Table 0-6, Table 0-7).

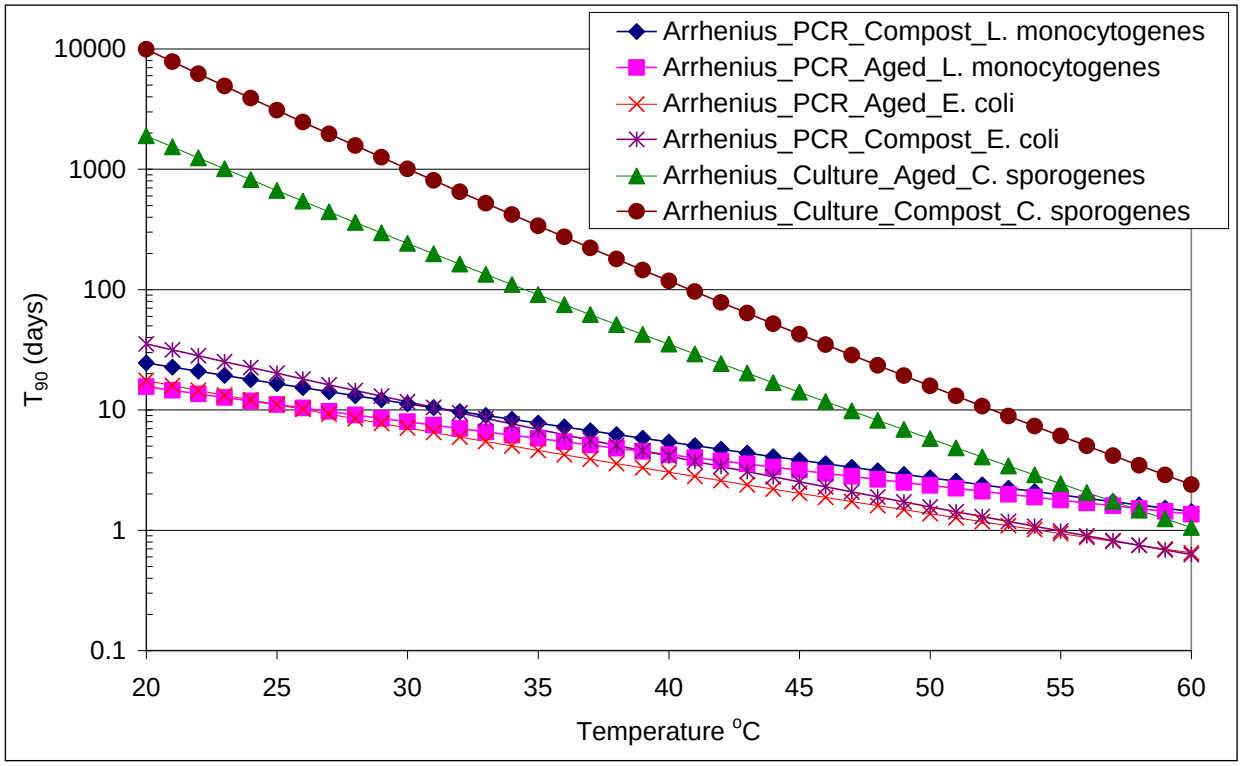
The effect of solar radiation on microbial inactivation in dust was also investigated (Appendix 21 Inactivation of Bacteria in Cattle Manure Dust in the Dark and by Solar Radiation). Some slow reduction was observed but it was not sufficient for radiation to be considered a barrier to exposure via the pathways identified.

Table 0-8. Inactivation rates (T_{90}; *k*) of inoculated *Escherichia coli*, *Listeria monocytogenes* and *Clostridium sporogenes* into microcosms with compost manure

Microorganism	Assay method	Temperature (°C)	n	T_{90}* (d)	k* (d^{-1})	R^2
E. coli	**qPCR**	20	21	27	0.086	0.85
		37	15	6.5	0.35	0.90
		50	9	1.7	1.4	0.99
	culture	20	18	4.4	0.53	0.97
		37	6	<0.9†	>2.6	0.99
		50	6	<0.3†	>7.8	0.99
L. monocytogenes	**qPCR**	20	21	65	0.035	0.61
		37	18	7.4	0.31	0.93
		50	12	2.5	0.93	0.94
		60	6	3.2	0.72	0.98
	culture	20	21	17	0.14	0.73
		37	9; 15	1.6; (121)‡	1.4; (0.019)	0.98; 0.26
		50	9; 15	0.60; 8.2‡	4.1; 0.28	0.98; 0.86
		60	6; 12	0.51; 14§	4.5; 0.17	0.99; 0.76
C. sporogenes	**culture**	20	15	185	0.012	0.39
		37	15	47	0.049	0.76
		50	15	10	0.22	0.97
		60	12	1.9	0.96	0.94

a. Statistical significance $P < 0.01$, $P = 0.01$-0.05 (in italics), or $P > 0.05$ (in parentheses).
b. † Results other than t = 0 less than the detection limit. Half detection limit values used for regression analysis.
c. ‡ 1st phase 0 to 6 days; 2nd phase ≥ 6 days.
d. § 1st phase 0 to 2 days; 2nd phase ≥ 2 days.
e. n = number of independent data values.

a



b

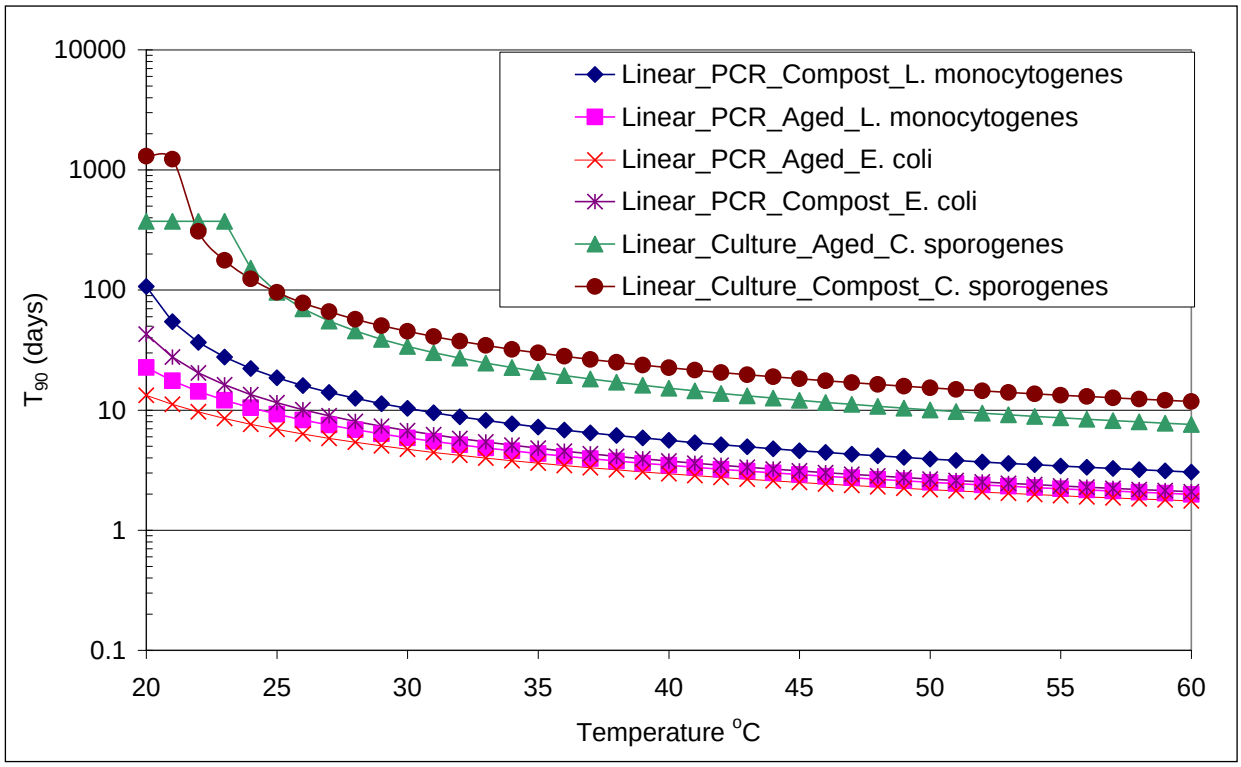


Figure 0-4. Conservative microbial Inactivation/Reduction as a function of time and temperature for different manures and microorganisms

a. Recommended algorithms based on Arrhenius curve fit.
b. Alternative algorithms based on Empirical 1: $a_0 + a_1*/^{o}K$ curve fit (see Appendix).

V.4.3 Aerosol Particulate Levels

The material forms in which different populations were most likely to contact contaminants were coarse dusts (which covered many feedlot surfaces) and fine aerosols (< 10 µm i.e. PM_{10}) especially during dry periods. Of these two, fine aerosols were seen as of more concern because of the potential for inhalation into the fine passageways and alveoli of the lung. With this in mind the survey of feedlot aerosols was commissioned from QUT. The primary report that was provided is reproduced in Appendix 22 Dust Measurements at Cattle Feedlots. In brief the objectives of QUT's sampling program were two-fold:

1. Conduct extended measurements (over 16–18 hours per day on several days) of ambient dust particle concentrations and size distributions in the centre of a well-managed cattle feedlot following a period of dry weather when dust levels were likely to be easily mobilised and hence relatively elevated. Sampling periods were to include the sundown 'frisky cattle' period.
2. Measure aerosol/dust emission from a variety of different short-term activities of varying scales.

The majority of measurements were taken at two feedlots in SE QLD- Feedlot#4 and Feedlot#2 during the week beginning 19 October 2009. Some limited measurements were also taken in the centre of Feedlot#4 during a very wet period on 7 September 2009. Conditions at the Feedlots at the time as well as aerosol generation are shown in Appendix 23 Feedlot Dust Emission Photographs.

The monitoring campaign was not designed to provide a comprehensive set of monitoring data sufficient to estimate long term risks. Rather it was designed to be a synoptic survey which characterized the main attributes of aerosols generated in feedlots and provides indicative estimates of their levels and emission duration on short (event) and medium (diurnal) timescales under worst case (dry) exposure conditions.

V.4.3.1 Ambient Feedlot Aerosol Concentration

Dust levels and their variance in the middle of Feedlot#4 are shown graphically in Appendix 24 Particle Emission Characteristics. Particle sizes were approximately evenly distributed in between the $PM_{2.5}$ and $PM_{2.5}$-PM_{20} fractions. The PM_{10} fraction accounted for approximately 70% of particles and this metric was used to estimate the numbers of particles (and mass assuming sphericity) which could be inhaled.

The majority of particles were fluorescent indicating despite their small size they contained viable microorganisms and were likely from the pad, cattle defecation or material exceeding rich in microbial biomass. Particle fluorescence was largely unchanged over the course of the day indicating the dust was mainly derived from the Pens as visually observed during the major emission peak between 1800 and 2000 which occurred due to increased cattle movement. During this latter period the concentration increased by 10 to 100 fold compared to other hours of the day (Figure 0-5). Windspeed during the monitoring period did not greatly influence the particle concentrations directly also suggesting that cattle movement was mainly responsible for these peaks. Thus for estimating risk under ambient conditions the material to which people were exposed was assumed to be pen manure. Further to this it was assumed to contain microbial cells in proportion to their levels in the different manure aerosols. This was seen as plausible based from the analytical experiments where the pad manure was found to be fine and easily dispersed when dry.

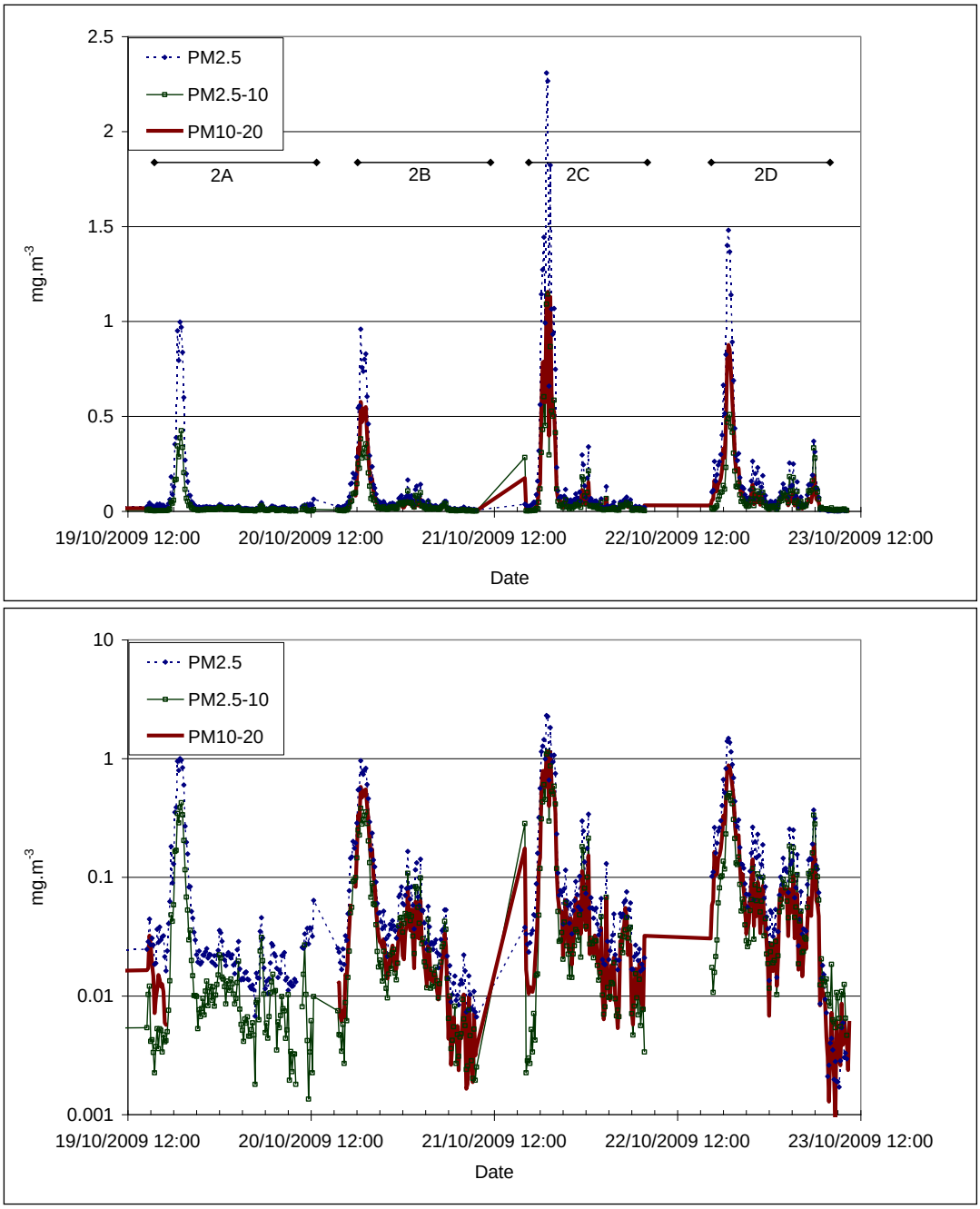


Figure 0-5. Timeseries of Particle Counts at Feedlot#4 Centre during Sampling Runs 2A, 2B, 2C, and 2D over 4 days.

V.4.3.2 Particle Concentrations during Events

For periods of a few hours on the 21/10 and 23/10 the particle monitors were moved from the centre of Feedlot#4 and repositioned immediately downwind of a variety of simulated and actual manure management activities to simulate extreme exposure events. In the majority of cases exposure to these activities was simulated repeatedly. The results are presented graphically in Appendix 25 Event Based Aerosol Particle Loadings. By in large typical background particle concentration was *ca* 0.01 $mg.m^{-3}$ and upwind dust often did not have a high total microbial content in contrast to measurements at the Feedlot centre.

Disturbances consistently generated dust concentrations downwind in the range of 0.1 to >1 $mg.m^{-3}$ whether activities were small, medium or large in scale. Total emissions were in the range of 0.3 to 800 $mg.s^{-1}$ and the PM_{10} fraction ranged from 30% to 80%. Event exposure

duration was estimated to be in the range of 10 s to 3 min. Older manure predominantly lacked a high microbial content supporting our conclusion that the highly fluorescent dusts seen during ambient aerosol monitoring were from Pen manure or relatively recently harvested manure. Events were classified as small, medium or large scale where:

- 'Small' related to dust generated by a single person moving or disturbing feedlot waste without mechanization e.g. using a shovel (as might occur during gardening as well as on-farm management).
- 'Medium' related to the dust generated during the use of mechanical equipment which was either small in size or only used for a limited period.
- 'Large' related to the dust generated during the use of mechanical equipment which created large visible dust clouds.

From this work event load statistics were tabulated as probability density functions suited to estimation of exposure concentration and exposure duration (Appendix 27 Dust Emission PDFs). The difference between 'medium' and 'large' was essentially one of value judgment.

V.4.4 Contaminated Run-off Water

Because the retention basins at feedlots are large and the run-off is generally captured and either evaporated or irrigated in a controlled manner, risks arising via exposure to run-off water were judged to be low provided water was not aerosolised and an extended storage time was allowed before reuse. However MLA and FSA agreed that some information should be obtained with a view to appropriate management. Appendix 26 Feedlot Run-off Bacteriological Quality during Significant Storms summarises the results of a synoptic survey of feedlot run-off water following a large regional rainfall event.

The survey was designed to evaluate what risk there might be from contact with the run-off during reuse or the impacts of discharge at times of very high rainfall (event recurrence interval > 20 years).

Levels of *E. coli* and enterococci in run-off were in the range of 10^6 to 10^8 .100mL^{-1} implying total emissions in the range of 10^{13} to 10^{15} indicators.Ha^{-1} of pad for the 24 and 72h events primarily from the pen manure which was probably the dominant source due to the high proportion of coverage. 13% of pad manure *E. coli* appear to be potentially pathogenic and at least 6 of the surveyed pathogens were detected in summer manure sampled from the 3 model feedlots. Overall the data indicated these pathogens were likely present in fresh run-off at levels of *ca* 10^3 to 10^5.L^{-1}.

We concluded it is essential to promote reduction in these numbers through several weeks storage and minimise the potential for contact by avoiding irrigation that promotes high aerosol production and drift.

V.5 Contaminant Intakes

Intakes considered were inhalation (PM_{10} particles) and ingestion (as dust). Intake estimates were based on:

- Contaminant concentrations in manures measured directly or estimated based on reduction due to inactivation in the case of pathogens
- Standard (USEPA) ingestion and inhalation rates.
- Aerosol particle sizing and concentration (exposure concentrations);
- Measured and conceptual estimates of exposure duration.

V.5.1 Standard Assumptions

Standard/baseline inhalation and dust ingestion assumptions considered are detailed in Appendix 28 Ingestion and Inhalation. The primary assumptions used in risk characterization were dust ingestion of 50 $mg.d^{-1}$ (central tendency) and inhalation at the long term rate of 16.3 $m^3.d^{-1}$ (mean). These values were used in all simulations for consistency. It should be noted that the 95^{th} percentile and moderate intensity inhalation rates are higher by factors of 2 to 3 but the differences are relatively small compared to other sources of variance.

V.5.2 Aerosol Exposure Scenarios

In the case of aerosolized dusts it was not possible to estimate overall exposure loads because of the diversity of possible exposure points and exposed populations. However, using the particle size data collected it was feasible to estimate doses of manure inhaled under a range of exposure scenarios reflecting:

1. Circumstances/events when exposure of different populations might occur;
2. The concentrations (and concentration variance) of manure derived aerosols to which they could be exposed under conditions of higher concern;
3. Durations of exposure at such times.

Four types of exposure scenario were developed as a basis for developing situation specific estimates of aerosol and dust intakes and hence risk estimation:

1. For feedlot workers and subcontractors exposed to ambient aerosols and dust a 7h working day duration was nominated and combined with ambient aerosol measurements and standard dust intake assumptions.
2. For visitors and feedlot workers and subcontractors exposed to aerosol peaks, a nominal 30 min transient exposure was evaluated noting the evening aerosol peak appeared to last *ca* 2 h.
3. In the case of simulated sporadic events arising from small, medium and large scale manure management and reuse activities, the duration of centre line downwind exposure was measured directly, and often repeatedly (up to 6 replicates). Duration statistics were compiled for use in risk characterisation.
4. Conceptual low, medium, and high exposure to sporadic plumes off-farm were defined conceptually as 5 s @ 0.01 $mg.m^{-3}$ (minimum aerosol level measured on-farm), 30s @ 0.1 $mg.m^{-3}$ (median aerosol level on-farm), or 5 min @ 1 $mg.m^{-3}$ (peak level of farm). These reflect the extreme ranges of exposure which could be experienced when travelling behind transport lorries in rural situations where dust is suppressed to varying degrees.

V.6 Uncertainties

Uncertainties in the exposure assessment included the following:

1. During aerosolization there was assumed to be no change in the microbial population numbers per mass of manure (based on measurements of microbial inactivation rates in dust).
2. The impact of high numbers of some pathogens or quantities of chemicals which might be encountered during hazardous events not modelled so far e.g. *S. enterica* during an outbreak;
3. Inactivation rates may have been somewhat different under different moisture conditions (Note that a. qPCR assays provided the main inactivation data and these were assessed to mostly likely be conservative and b. the moisture content of materials studied were comparable to material measured in the field).
4. The (conservative) assumption that dust measured at the feedlots was primarily derived from different manures. (This assumption was considered reasonable during the evening dust peaks and in the dust plume generation experiments where particle

counts were undertaken against a low background (*ca* 0.01 $mg.m^{-3}$). Further during the day the fluorescence of particles did not alter dramatically indicating it was predominantly from the same source).

5. How representative / conservative our worst case conditions were of aerosols over the long term.
6. The conceptual nature of some of the scenarios, especially manure transport off-farm.

Exposure to contaminated run-off was not analysed in detail due to resource constraints. However bacterial indicator levels in the ponds were measured immediately after a storm event and 1 week further on. T_{90}s of *ca* 2 days were observed indicating storage of a few weeks should reduce the loads of pathogens sufficient to minimize risks from irrigation.

VI Risk Characterization

VI.1 Summary of Risk Characterization Process

The risk characterization process is summarized in Figure 0-6. In summary the process was as follows:

1. Exposure scenarios (aerosols and dusts) were constructed reflecting real world conditions and describing how contaminants in primary waste materials would be ingested or inhaled i.e. cause and effect paths by which populations are exposed (The Exposure Scenarios for which risks which were quantitatively assessed are listed in Table 0-9.).
2. Levels of contaminants in each type of carrier material (mainly manure dusts) were estimated:
    a. directly from the survey; or
    b. by combining primary survey data and inactivation/decomposition rate estimates; or
    c. as an upper limit value or average based on assay sensitivity considerations and the number of samples assayed.
3. Concentrations of dust/manure to which populations might, or could, be exposed were:
    a. measured experimentally (ambient and event based); or
    b. estimated conceptually reflecting experimental measurements.
4. Dust/manure concentration data and manure contaminant content data were combined to estimate airborne contaminant levels.
5. Using dust contaminant level data and standard inhalation and ingestion rate data, exposure intakes were calculated.
6. Exposures typically considered were a 7 h working day, a 30 min short encounter, or a transient exposure during events (5 s to 5 min).
7. In each scenario one exposure was assumed to occur per day.
8. In the case of each pathogen, literature dose response algorithms are used to calculate a risk rating based on estimation of probability of infection.person^{-1}.exposure^{-1}.
9. In the case of chemicals intake risk was assessed using a rating system based on Hazard Quotient estimates.

Constraints on the numbers of exposure scenarios which risk could be modelled and risk assessment more broadly included:

- Combinatorial explosion (the numbers of credible exposure scenarios which can be constructed is so great that exhaustive risk assessment is impractical).
- There are not as yet agreed dose response relationships for some of the pathogens and chemicals (in these cases reasonable approximations and assumptions were used).

This led to risk estimation involving modelling of the selected set of the exposure scenarios outlined in Table 0-9 and detailed in Appendix 29 Exposure Scenarios.

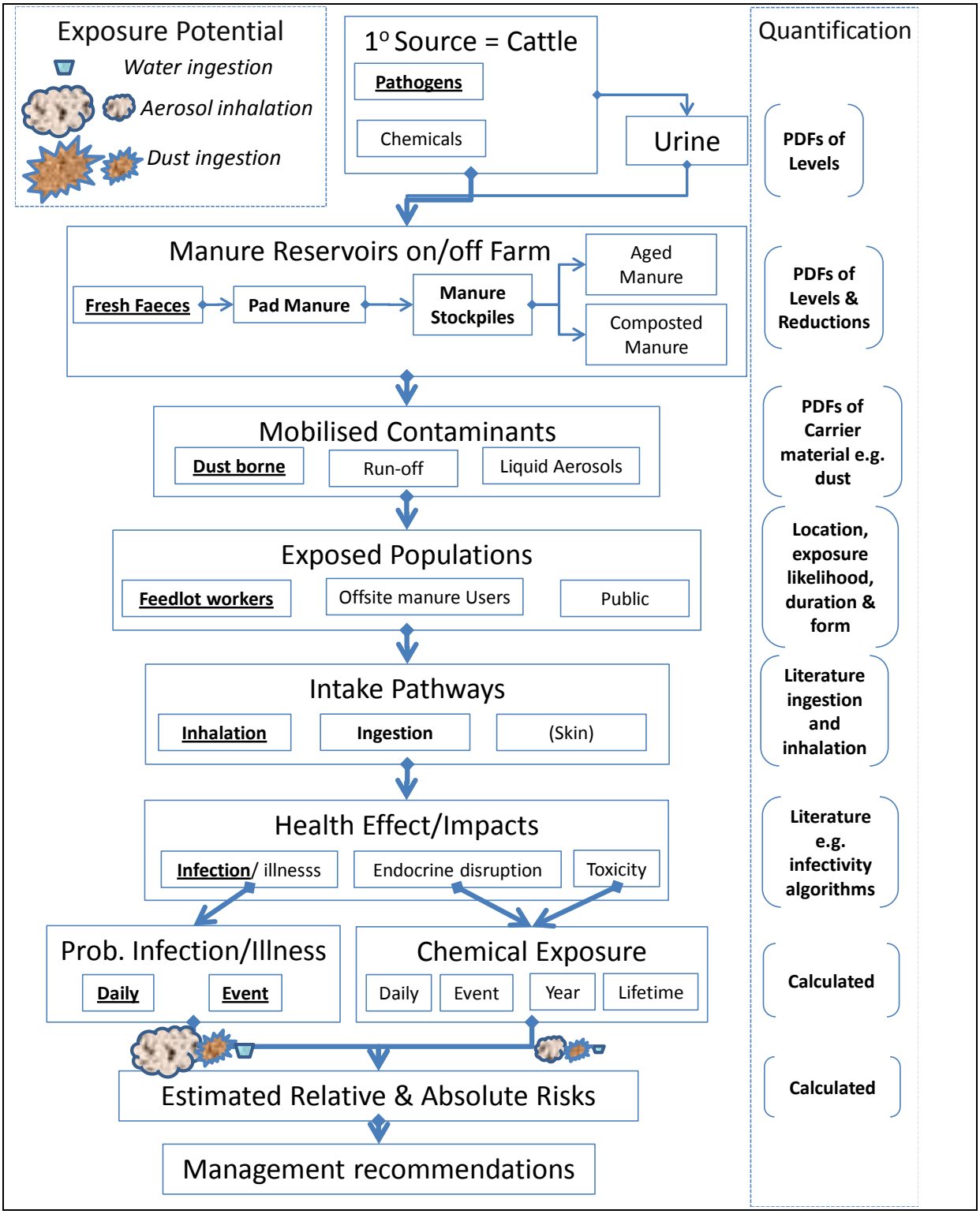


Figure 0-6. Generic Risk Characterization Process

a. Relative potential exposure is illustrated by the size of the icons
b. Bold and underlining emphasize highest concerns

Table 0-9. Summary of Exposure Scenarios Modelled

Exposure Scenario Group	Issue No.s	Selected Details	Waste Material	Comments	No. of pathogens/ Assessment of Chemicals[a]
Ambient Pen Manure Dust inhalation	1-3	Site Workers and visitors (30 min to 7 h exposure)	Pad manure	PM_{10} inhalation	14/++
During Management	4,5	Site Worker Pad Manure Intermediate Dust Level (0.1 $mg.m^{-3}$, 7 h exposure)	Pad manure	Conceptual – to assess safety of specifies aerosol levels	9/++
Ingestion of Pad Manure Dust	6, 7	Workday Pad Manure Dust Ingestion (7 h exposure)	Pad manure	Median values	9/++
Ingestion of Compost Dust of Harvest Manure after composting	8,9,10	Composted Dust Ingestion (7 h exposure)	Composted manure	Chemical assessment covered by other scenarios	9
Aged Manure & Composted Trucks Producing Aerosol for Motorist	11, 12, 13 and 17, 18, 19	Motorist Aged Manure Truck High Dust 0.01-1 $mg.m^{-3}$ (5s to 5 min exposure)	Aged manure or composted manure	Conceptual	9/++
Ingestion of Aged Manure	20, 21	Aged Manure Dust Ingestion (0.5 to 7 h exposure)	Aged manure	Chemical assessment covered by other scenarios	9
Small Event Exposure	22 to 32	small scale Activity 1	Aged, composted and harvested manures	Various scenario simulating small activities such as shovelling compost in a garden setting.	4/++
Medium Event Exposure	33 to 48	medium scale Activity 22	Aged, composted and harvested manures	Various scenario simulating medium scale activities such as bobcat scraping of pens	4/++
Large Event Exposure	49 to 52	large scale Activity 6	Aged manure	Various scenario simulating medium scale activities such as spreading on fields	4/++

a. Chemical Assessment is shown as '++'. Not all scenarios were assessed as the contaminant levels were very low and the same exposure assumptions (high levels) were used for different manures.

VI.2 Risk Quantification

VI.2.1 Risk Benchmarking

Quantifying risk was seen first as providing MLA stakeholders with estimates of **Relative Risk,** and a rationale for waste management resources might be best used with health risk minimization in mind where a number of alternate options were mooted or identified by other means (e.g. via qualitative assessment). The second use of risk quantification was seen as providing MLA's stakeholders with estimates of **Absolute Risk,** that is the actual likelihood of populations exposed to feedlot contaminants contracting illness. Conceptually knowing the magnitude of risk informs the decision of whether management is needed at all, how urgent the need for management is and how actual risks compare to pre-agreed standards or **Benchmarks** where such existed. Though the calculation and use of absolute risks is conceptually straightforward, in practice estimation of a credible risk estimate or PDF can be more challenging for a variety of reasons where a new hazardous situation are being assessed. Considerations involved in calculating risk and assessing risk estimates against benchmarks are detailed in Appendix 30 Risk Benchmarking Considerations.

Provisionally for pathogens we have used the risk assessment scheme summarized in Table 0-10 where estimates of arithmetic average infection probability are used to estimate textual and numerical risk ratings. It is emphasized that the primary use of the ratings was to provide a clear and consistent basis for identifying elevated risk situations and prioritizing risk management actions rather than to provide an estimate of total absolute risk. Further information explanation of the system is provided in Appendix 31 FLOT 333 Risk Benchmarking.

Table 0-10. Proposed Pathogen Risk Rating Categories for Scenario Exposure Risk

Risk Description Used in Text	Numerical Risk Rating ($-\log_{10}$ of infection probability)	Scenario Management Implications
Very Low	>4 to 10	Risk appears very low especially when conservative input assumptions are recognized. Risk should not be discounted but situation concerns appear to be of relatively low.
Low	4	Risk is relatively low but mitigation possibly needed especially if exposure is repeated.
Moderate	3	Moderate risk. Management of exposure probably required.
High to Very High	0 to 2	High to very high risk is indicated. Management/ exposure mitigation required.

In the case of the Chemical contaminants Hazard quotients (HQ) were determined for all exposure scenarios based on the ratio of the predicted 'upper-limit' level of exposure to predetermined safe levels of exposure. All HQs were determined according to the following calculation:

$$HQ = \frac{\text{Concentration } (ng.g^{-1}) \text{ x intake rate } (g.h^{-1}) \text{ x intake time } (h.day^{-1})}{\text{ADI } (\mu g.kg(bw)^{-1}) \text{ x } 60 \text{ kg x } 1000}$$

The calculation of HQs normally includes the use of uncertainty factors (UF) to account for uncertainty in the use of toxicological data. However, it is noted that such appropriate UFs have already been included in the determination of Acceptable Daily Intakes (ADIs) from original toxicology data.

Interpretation for HQ estimates for risk management was based on the scheme in Table 0-11. For consistency with pathogen assessment the HQ values have been converted to negative logarithms.

Table 0-11. Proposed Chemical Risk Rating Categories for Scenario Exposure Risk Assessment

Risk Description Used in Text	Risk Rating ($-\log_{10}$ of HQ)	Scenario Management Implications
Acceptable	>0	Exposure does not exceed acceptable daily intake.
Not acceptable	<0	Exposure may exceed acceptable daily intake (given conservative assumptions applied in model calculations).

Further details on HQ estimation and use are provided in Appendix 32 Chemical Risk Benchmarking.

VI.2.2 Model Input Assumptions

Pathogen risk estimation involved the selection of:

1. Exposure scenarios;
2. Aerosol particle concentration PDFs (and in the case of events their duration);
3. PDFs defining pathogen concentrations in aerosols and dusts based on pathogen concentrations measured in the different manures and their potential inactivation during aging or composting;
4. Air inhalation and dust ingestion rates;
5. Dose response algorithms.

Chemical risk assessment involved a comparable approach:

1. Exposures as ng.person^{-1}.d^{-1} were calculated and tabulated;
2. Acceptable Daily Intake values were acquired from the literature;
3. HQs were calculated as the ratio of highest likely dose to Acceptable Daily Intake dose (see above);
4. The HQ was transformed ($\log_{10}$ 1/HQ) to produce a second logarithmic scale.

Details of the assumptions are provided in Appendix 33 Primary Input Assumptions for Estimation of Risk. Calculations were undertaken using an Excel spreadsheet program and the @Risk 4.5 add-in. The program was designed as a metamodel so that further exposure scenarios could be modelled in the future.

The final pathogen risk ratings are compiled as Pi vot Tables (Appendix 35 Pathogen Risk Ratings for Aerosol Inhalation and Dust Ingestion Scenarios). Details of how to read these are provided in Appendix 34 Pathogen Risk Rating Tables.

Chemical risk rating tables are shown in Appendix 36 Chemical Hazard Ratings (-log10 HQ) for Steroidal Hormones and Ectoparasiticides.

VI.3 Risk Characterization Outcomes

VI.3.1 General Observations

All pathogens (and indicators) assayed for were detected many is substantial numbers. Most chemicals of potential concern were detected albeit in low concentrations. In the case of parasiticides they were totally absent from some feedlots most likely because they were not used in stock rearing.

The high numbers of pathogens in raw faeces most notably the abundance of EHEC_EPEC *E. coli* and *Campylobacter* suggested generally pathogen exposure posed a greater risk than chemicals. However, pathogens were generally more labile than chemicals and there was significant opportunity for reducing their numbers to tolerable levels.

The levels on contaminants observed in different manures appeared consistent with literature reports. Exposure assessment indicated inhalation of contaminated aerosols and dust ingestion were the intake routes of most concern.

The overall qualitative picture that emerged was that there were potential risks from all analytes but which risks were of most concern and what were the absolute risk levels was unclear. We concluded that to obtain sufficient information to support credible management recommendations it would be necessary to quantify risk as had been proposed at the project's commencement while noting the many uncertainties arising from the input data outlined in the Hazard Assessment and Exposure Assessment.

With this in mind some 338 QMRA and 663 QCRA distinct risk scenario simulations were modelled (@Risk 4.5) covering all pathogens or those of greatest concern, and all chemicals of potential concern.

VI.3.2 Pathogen Risks

The most abundant pathogens were:

- The Enterohaemorrhagic/ Enteropathogenic (EHEC_EPEC) *E. coli* group.
- *Listeria monocytogenes*
- *Campylobacter jejuni*
- *Cryptosporidium parvum*
- *Giardia lamblia*

Less abundant but still sporadically detected, and hence still a concern, were:

- *Salmonella enterica*
- *Yersinia pseudotuberculosis*
- *Leptospira spp.*
- *Coxiella burnetii*
- *Mycobacterium paratuberculosis*

Leptospira and *Coxiella* are considered highest risk pathogens (laboratory containment level required for experimental work needs to be > P2). They were only detected sporadically at or near the assay detection levels. Nevertheless qPCR assay sensitivity typically of the order of on 10^{-2} to 10^{-3} gene copies.g^{-1} indicated that a quantity of dust as little as a few milligrams could contain sufficient organisms to lead to infection. Thus sporadic detection was still a concern and full QMRA using the limited data available was seen as justified.

The most hazardous material was the pad manure. This was as a consequence of:

- The elevated levels of pathogen present compared to the other manures and its continual amendment by cattle with fresh faeces (demonstrated by the pathogen survey).
- Its total load/surface area covered was highly erodible leading to leading to a high potential for contaminating run-off and the atmosphere.
- The capacity for finer particles to be aerosolized during dry weather;
- Cattle activity generating dust clouds due to increased activity in the early evening.

Once stockpiled or windrowed, manure contaminants posed much less of a risk but pathogens were still present and relatively frequently detected. Thus aged material and compost still needed to be handled with care.

Carcass composts were not found to have an especially high content of pathogens compared to other aged and composted manures.

Quantitative Risk assessment was performed using Monte Carlo techniques which integrated:

- Source data (pathogen and chemical levels in different manures);
- Inactivation rates (pathogens only);
- Manure aerosol concentration data;

- Inhalation and ingestion rates;
- Dose response algorithms.

The average risk ratings from each scenario are detailed in Appendix 35 Pathogen Risk Ratings for Aerosol Inhalation and Dust Ingestion Scenarios. The primary findings and conclusions arising are summarized in Table 0-12

Table 0-12. Summary of Characterized Pathogen Risks

Issue	Findings and Observations	Conclusions
On-farm Pad Manure Dust inhalation	• The high dust levels measured combined with the high pathogen content of pad manure assessed in the survey to generate a number of *High* risk ratings. • Relatively *High* ratings were seen not only with site workers but also visitor exposure. • *High* risk ratings were also estimated when low and intermediate dust levels were assumed.	• Recognizing that pen manure dust aerosol generation is probably inevitable at any feedlot during dry weather, active management of exposure to pen manure dust is still probably needed. • Generic options include: o avoidance, especially of the evening peak; o hygiene education; o making protective devices available; o wetting of pad surfaces • Management actions should be targeted at all feedlot workers and visitors.
On-farm Dust Ingestion	• As with inhalation several pathogen in pen manure appear to pose a *High* risk. • Working with Aged manure for an extended period of time also appears to pose a relatively *High* risk. • Robust composting sufficient to reduce pathogen numbers by 5 orders of magnitude reduce risk ratings to a *Low* to *Very Low*.	• Pen manure should be actively managed. • Aged manure can still contain a range of pathogens at levels of concern which require management. Export off-site needs to consider how exposure to downstream users should be controlled. Simply aging manure prior to export may not be sufficient. • Composting or equivalent effective pasteurization should be able to achieve a *Low* to *Very Low risk* rating even in the event of exposure over an extended period of time.
Inhalation of dust during Small Scale Activities On-farm	• All risk ratings were estimated to be *Low* or *Very Low.* • The exposure conditions explored were worst case i.e. immediately and closely downwind of the activity modelled.	• Transient exposure to small dust plume events of duration 10 seconds to a few minutes does not appear to pose a problem (mainly aged manure and composted manure).
Inhalation of dust during Medium to Large Scale Activities On-farm	• There was no clear difference between events classed as medium and large so the two are considered here together. • Risk ratings for Aged manure and composted manure were generally estimated to be *Low* or *Very Low.* • However some ratings for harvested manure were estimated to be *Moderate* to *High*. • The exposure conditions explored were worst case i.e. immediate and intimate exposure downwind of the activities modelled.	• Transient exposure to dust plume events when managing harvested manure appears to require active management comparable to that for pen manure dust management (avoidance, education etc.).
Off-farm dust ingestion	• Working with Aged manure for an extended period of time also appears to pose a relatively *High* risk. • But robust composting sufficient to reduce pathogen numbers by 5 orders	• Aged manure can still contain a range of pathogens at levels of concern which require management and export off-site needs to consider how exposure to them should be subsequently controlled.

Issue	Findings and Observations	Conclusions
	of magnitude reduces risk ratings to a *Low* to *Very Low*.	• Composting or equivalent effective pasteurization should be able to achieve a *Low* to *Very Low risk* rating even in the event of exposure over an extended period of time.
Off-farm Inhalation of Small Quantities of Manure and Compost Dust During Events	• The risks considered have risk ratings to a *Low* to *Very Low*.	• Short term exposures were assessed as having short term risk ratings of *Low* to *Very Low*.
Inhalation of Dust During Aged Manure and Compost Transport	• Transporting compost appeared to pose *Very Low* risk from short duration fugitive emissions. • Transporting aged manure posed a *Low* to *Very Low* risk unless exposure was both extended and the aerosol concentration was high.	• During transport aged manure and composted manure should be covered. The occasional fugitive emission does not appear to pose a substantial risk. • This may not be applicable to fresh pen manure and its early transport off-site is not recommended.

VI.3.3 Chemical Risks

Chemical risk estimates are documented in Appendix 36 Chemical Hazard Ratings (-log10 HQ) for Steroidal Hormones and Ectoparasiticides. All had ratings > 0 (i.e. HQ <1). It was concluded that the overall risk posed by the ectoparasiticides and steroidal hormones to the populations were low to negligible even under the high exposure scenarios considered. Actions designed to reduce pathogen exposure should further reduce any residual concerns regarding chemicals.

VII Risk Management Recommendations

Based on the characterized risks producers guidelines have been developed (Appendix 37 Guidelines for the Safe Management of Feedlot Wastes). Further to this provisional recommendations are presented below in dot point.

The producer guidelines take into account best practice and current industry practice. These guidelines should be seen as living documents to be updated as industry waste management expertise develops and in light of new information that emerges in the future.

VII.1 General

- Risk management recommendations should be applicable to all feedlots irrespective of state or geographic locality.
- Risk management recommendations are directed at protecting against pathogen risks except where indicated.
- MLA should promote/establish systems to ensure:
    - awareness that manure, pen manure in particular, has significant numbers of all 10 zoonotic pathogens surveyed including the EHEC_EPEC group, *Campylobacter,* and *Cryptosporidium*;
    - awareness that the pathogens pose a range of risks, in the first place gastroenteritis (e.g. EHEC_EPEC, *Campylobacter*, *Giardia*), but also can cause other diseases/sequelae (Q Fever, Leptospirosis);
    - awareness that processed manure wastes contain reduced but still significant numbers of pathogens and material must be treated accordingly;
    - good hygiene among all staff especially pen workers and other outdoor staff;
- Pregnant women should be made aware that there is a high numbers of *Listeria monocytogenes* in the pen manure even though their infectivity appears to be low.
- Procedures developed in the water industry for handling and recycling biosolids safely may in principle be probably appropriate for application to manure.
- Fine aerosolized dusts generated by wind, cattle and feedlot activities during dry periods appear to pose the greatest risk.
- During dry periods consideration might be given to wetting / dust suppression of the pad manure.
- Use of run-off water is not recommended on first principles without treatment (chlorine? Economics?).

VII.2 Protection of On-Farm Staff

- Avoid/minimize:
    - pen riding during high aerosol generation periods (winds, 6-8 pm cattle activity period)
    - standing downwind of any aerosol generating activity for any length of time without protection.
    - moving/transporting pen manure dust when it is dry. Consider wetting the material or moving/mounding when it is damp e.g. early morning or after light rain.
    - working outdoors generally on dry windy days.
- Provide high quality (P2) dust masks where requested.
- Develop criteria for what constitutes ‘unacceptable’ aerosolized dust exposure.
- Develop an industry standard definition for what dry windy days entail as a basis for triggering the above actions.
- Explore introducing dust monitoring equipment ($PM_{2.5}$ or PM_{10}) and incorporation into current routine monitoring.
- Offices should be fitted with air filters to protect staff.

VII.3 Manure Management on-site

- Current on-farm management practices (windrowing, separation, treatment) are appropriate in principle but probably need to be standardized to minimize risk and maximize effectiveness of pasteurization/inactivation process (e.g. windrow temperature, storage time prior to reuse).
- Before export off-site or reuse, composting or other disinfection treatment is strongly recommended. Simply storing in windrows for an arbitrary period is insufficient (Composting appears current method of choice in Australia and reasonable given land availability in most localities).
- It may not be necessary to amend manure prior to composting provided sufficient carbon remains in the harvested manure to achieve high temperatures (> 50^{o}C) as currently undertaken at Feedlot C.
- Brief exposure to small dust plumes from aged manure and compost were risk rated at >3 i.e. *Low* risk. Thus infrequent sporadic exposure should not be a major concern but repeat exposure should be avoided.
- Brief exposure to large dust plumes from aged manure and compost were also rated at 3 to 4 i.e. *Low* risk. Thus similarly infrequent sporadic exposure should not be a major concern but repeated or extended exposure should be avoided.
- Exposure to harvested manure was rated in several instances at *Moderate* to *High*. This indicates the need to minimize dust generation and exposure during harvesting of pen manure and initial preparation of windrows. This could include covering of loads and avoidance of the harvesting areas without protection.

VII.4 Irrigation of Run-Off Water

- Pen run-off has a very high indicator and probably pathogen content. This needs to be actively managed.
- Contamination levels are such that irrigation onto pasture or crops must be done only when the catchment is dry and no rain is forecast and the soil profile is dry. It is recommended that this be done after there has been sufficient opportunity for indicator/pathogen reductions.
- T_{90}s of *ca* 2 to 4 days were observed with *E. coli* and enterococci.
- Following significant run-off inputs it is suggested that a minimum of 2 weeks HRT be allowed to ensure reductions in resilient pathogens. Preferably allow 4 weeks before reuse.
- Avoid using high pressure aerosol generating irrigation systems.
- Well designed (i.e. flow well distributed) flood irrigation.
- Avoid working downwind of spray.
- Avoid irrigation on windy days or when ground is wet.

VII.5 Miscellaneous

- Pen manure dust on machinery, fences and roads should be treated with care and may be a concern in the days after its initial deposition e.g. up to *ca* 1 week after a gale. Thereafter solar radiation should disinfect surface dust deposits (this should/could be verified experimentally using indicators).

VII.6 On-farm Visitors

- Visitors should be made aware of the hazardous nature of the different waste materials via an information sheet.
- Anyone with medical condition reducing their immunity, or pregnancy, in particular should be discouraged from visiting or working at Feedlots.
- Children should not be allowed on-site.

VII.7 Off-farm Manure Reuse/Users

- As for on-farm visitors:

Also:

- Manure spreading on cropping fields should not be undertaken during high winds.
- Dust generation should be minimized.
- Spinning disc type spreaders are not recommended without dust management e.g. ensuring moisture content sufficient to suppress fine aerosol generation.
- Prior to export off-site waste manure should be composted in line with current best practice Guidelines established for other industries especially for biosolids reprocessing.
- Wastes for export should be windrowed for sufficient time to reduce pathogen numbers by at least 3 and preferably 5 $\log_{10}$s. This is likely to be 2 to 4 months. Effectiveness may vary with season so verification of local operating practice is recommended. If a short storage or stockpiling is proposed evidence as to the safety of the material should be acquired e.g. indicator monitoring, or temperature records showing that sufficient pasteurization has occurred.
- Ensure large scale re-users are aware of residual hazards associated with manure/compost and its reuse and options for exposure avoidance.
- For small scale re-users ensure:
    - Any commercially sold material has appropriate warning signs.
    - Summaries of disinfection / processing are available.
    - (possibly) Microbiological quality control test report data.

VII.8 The Public

VII.8.1 Motorists

- Trucks used to transport bulk composted manure and aged manure off-site by public roads must be covered to prevent aerosol production.
- Small quantities of fugitive emissions should not pose a *High* risk.

VII.8.2 Neighbours

- Bearing in mind the emissions of Pen manure the risks arising probably need further risk assessment.

VII.9 Composting and Aging

- The T_{90} required for inactivation of Gram negative and Gram positive microorganisms at 35^{o} C based on qPCR and culture methods was < 10 days for both aged manure and composted manure. Thus composting for 2 months should be sufficient to achieve a 5 $\log_{10}$ reduction for typical vegetative cells should be sufficient provided elevated temperatures are maintained and the manure is satisfactorily mixed. Higher temperatures can achieve still fast inactivation. These criteria apply to the most abundant pathogens notably *E. coli* and *Campylobacter.*
- The T_{90} required for resilient microbial indicators such as spores in some experiments exceeded 10 days at temperatures < 50 ^{o}C. Thus to ensure potentially resilient pathogen cell forms (e.g. *Coxiella*, *Mycobacteria, Cryptosporidium*) were reduced sufficiently a longer time may be required if these are suspected of being abundant.
- Alternatively given that compost windrows undergo a sharp elevation in temperature followed by a gradual decline an alternative may be determine based on the time temperature dependent relationships calculated in the project whether the degree of inactivation in a given compost system was sufficient for end product re-use.

- The effectiveness of composting or aging should be verified via tests for both *E. coli* and enterococci (assume use of Enterolert^{tm} and Colilert^{tm}) to assess whether numbers are < 10 mpn.g^{-1}.
- If there is still concern additional monitoring could be undertaken to determine whether:
    - There are no detectable resilient organisms remaining e.g. sulphite reducing Clostridial spores;
    - There are no pathogens detectable by qPCR (limitation is assay sensitivity of *ca* 10^3.g^{-1} manure where enrichment is not possible).
- The most cost effective monitoring to emphasize is probably to monitor representative windrows using thermistor chains.

VII.10 Composted Carcasses

- Carcass compost was not observed to be any worse in contaminant content than normal compost. Thus this method for carcass disposal seems reasonable provided the loading is not unusually high or the suspected reason for the mortality does not involve a pathogen e.g. *S. enterica*. If this is the case the recommendations in the previous section would seem applicable.
- In the event of disease being cause of death, or if there are any doubts for other reasons, then monitoring of *E. coli* and enterococci levels (target being no detectable indicators) should allow assessment of whether inactivation has been sufficient prior to such materials being recycled into Feedlot fields. Where a specific pathogen of concern has been identified this could also be assay for using PCR technology.

VII.11 Reuse

VII.11.1 Broadacre Agriculture

- The loads of pathogens which are deposited by cattle onto agricultural fields under normal conditions (e.g. grazing, dairy) should be much greater than the greatly reduced load which should be achievable in composted / aged manure(nominally recommended as 5 orders of magnitude compared to harvested manure).
- Thus the use of manure for broadacre agriculture seems reasonable.
- To minimize any run-off of residual pathogens, application should avoid periods of cold overcast weather/rainfall by consulting weather forecasts (several days solar radiation onto scattered manure fertilizer should further reduce residual pathogen levels provided material clumps are not large.
- A possible constraint may be not human but rather ecological health impacts of residual parasiticides which might need to be assessed for whether soil application leads to sufficient dilution (The survey included measurements of parasiticides levels in manures where these were used which could be combined with helminth toxicity data.)

VII.11.2 Horticulture and Organic Farming

- The main constraints on reuse in horticulture and organic farming appear to be:
    - Ensuring pasteurization has been effective.
    - Ensuring residuals are not present at levels of potential concern

VII.12 Monitoring of Aged Manure and Composted Manure Quality

- Normally there would be no reason for monitoring fresh faeces, pad manure or harvested manure.
- In the event that there is concern over pathogens in Feedlot herds, that there were higher numbers of one or more of the pathogens that observed in this study (or indeed others) qPCR of fresh faeces or pad manure (included urine) appears to be sufficient sensitive to assess the levels of initial contamination and follow their fate (e.g. in a fallow pen)

- In the case of wastes for export or to be reused on-site in locations where run-off could mobilize the material and pollute surrounding waterways
- Recommended approaches are:
    - Monitoring and documenting windrow age systematically.
    - Monitoring and documenting windrow temperature using a thermistor string to ensure:
        - Pasteurization temperatures sufficient inactivate pathogens are achieved and maintained for a sufficient time to inactivate pathogens of concern (can be assessed using the algorithms developed in this project);
        - Pasteurization occurs through the whole of a windrow or compost heap (windrowing without mixing is insufficient)
    - On maturation, composts or aged manure should be tested using representative samples (exterior as well as interior of piles) for both *E. coli* and enterococci as the former can be relatively easily inactivated and there were virtually no false positive samples containing pathogens where neither indicator was present.

VII.13 Future Work

- Composting without amendment (convenient)
- Rapid pasteurization followed by immediate reuse (could reduce land take and loss of nitrogen; immediate reuse could retain nitrogen status and reduce pathogen regrowth potential).
- Establish long term dust monitoring to ascertain severity of problem.
- Risk benchmarking involving government and industry stakeholders and confirm provisional approach developed here.
- Complete aerosol transport work originally planned by WRC/FSA to understand scale of risk to neighbouring properties.
- Identification of compost needs (as interim measure Biosolids recommendations should be used).
- Parasiticide impact on the natural environment.
- Initiate discussions with environmental health at national level to avoid over-reaction to high risk estimates.
- Develop dust suppression methods.
- MLA recognize that the issues/situation studied:
    - May apply in the case of dairies as well;
    - May apply in the case of piggeries as well;
    - Discuss how to develop guidelines.

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