



**Australian Government**

**Department of Agriculture, Fisheries and Forestry**

*Advice Note for the National Consultative Committee on  
Animal Welfare*

**Biotechnology, genetic modification, cloning and  
animal welfare**

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## ACRONYMS AND ABBREVIATIONS

<b>AAWS</b>	The Australian Animal Welfare Strategy
<b>B-D:</b>	Biotechnology-derived
<b>CAC:</b>	Codex Alimentarius Commission; a joint agency of the FAO and WHO
<b>CFIA:</b>	Canadian Food Inspection Agency
<b>DNA:</b>	Deoxyribonucleic acid
<b>EC:</b>	European Commission
<b>FAO:</b>	Food and Agriculture Organization of the United Nations
<b>FAWC:</b>	Farm Animal Welfare Council of the UK
<b>GM:</b>	Genetically modified
<b>NCCAW</b>	National Consultative Committee on Animal Welfare
<b>NRC:</b>	National Research Council of the USA
<b>UK</b>	Department of Environment, Food and Rural Affairs of the United Kingdom
<b>DEFRA:</b>	Kingdom
<b>USDA:</b>	United States Department of Agriculture
<b>WHO:</b>	World Health Organization of the United Nations

## GLOSSARY

<b>Artificial selection</b>	Purposeful selection for breeding by humans of animals with genetic characteristics that are regarded as favourable and which lead to an increase in individuals with these favourable characteristics in succeeding generations.
<b>Biotechnology</b>	The application for industrial purposes of scientific, biological principles. In particular, the use by industry of recombinant DNA, cell fusion, and new bio-processing techniques.
<b>Blastocyst</b>	A stage in early embryonic developments in which the cells form a sphere with a fluid-filled cavity in the centre.
<b>Breeding technologies</b>	Breeding methods other than natural mating used to intensify genetic selection.
<b>Blastomere cell nuclear transfer (BNT)</b>	Nuclear transfer in which a nucleus from a blastomere (that is, a cell produced when the fertilised egg or zygote undergoes cell division) is transferred to an enucleated egg cell. See also somatic cell nuclear transfer (SCNT).
<b>Cell</b>	The smallest unit that may possess all the properties associated with life. Usually consists of one or more nuclei surrounded by cytoplasm and enclosed in a membrane.
<b>Chromosome</b>	A threadlike structure of DNA and associated proteins which is found in the nucleus of a cell. Chromosomes carry genetic information in the form of genes.

<b>Cloning</b>	In its usual sense, cloning refers to the propagation of genetically exact duplicates of an organism by means other than sexual reproduction. The term cloning has been assigned to the reproductive technology of somatic cell nuclear transfer. Progeny obtained from somatic cell nuclear transfer are genetic near copies, not genetic replicas, of the somatic cell donor.
<b>Conventional breeding</b>	Breeding methods based on natural mating.
<b>DNA</b>	Deoxyribonucleic acid, which is present in almost all living cells and contains information coding for cellular structure, organisation and function.
<b>DNA construct</b>	A DNA sequence that has been modified for the purposes of transgenesis.
<b>Embryo</b>	In mammals, the stage of development between the time the blastocyst attaches to the wall of the uterus and the onset of the foetal period when the major features of the external body form become visible. In cattle, this is about day 45 of gestation.
<b>Enucleated cell</b>	An egg cell or oocyte from which the nucleus has been removed by mechanical means.
<b>Epidemiology</b>	The study of the distribution and determinants of health-related problems or events is specified populations of people or animals. The term can be extended to welfare- related problems in animal populations.
<b>Epigenetic inheritance</b>	Inheritance through alterations in DNA function without alterations in DNA sequence; that is, transmission of information from a cell or multicellular organism without that information being encoded in the nucleotide sequence of the gene.
<b>Epistasis (epistatic adj.)</b>	Gene interaction and, particularly, interaction between different alleles at different genes. Epistasis can occur at the same step or at different stages of the same biochemical pathway.
<b>Evidence-based policy</b>	A rigorous approach to the development of policy that draws on careful data collection, experimentation, and both quantitative and qualitative analysis to answer relevant questions.
<b>Founder effect</b>	A phenomenon in which newly established populations more closely represent the genetics of their founders than the overall genetics of the population from which the founders came.
<b>Gene</b>	Genes are the biological basis of heredity and occupy precisely defined places on chromosomes.
<b>Genetic code</b>	The orderly arrangement of nucleotide units in molecules of DNA that carries the genetic information in living cells.
<b>Genetic diversity</b>	The range of genetic variation present within a population.
<b>Genetic drift</b>	The process whereby the relative frequencies of genes changes randomly from generation to generation as a result of the small size of the breeding population.

<b>Genetic modification</b>	Refers to the process whereby the genotype of an organism is modified by the application of biotechnology.
<b>Genetics</b>	The study of inheritance.
<b>Genomics</b>	The study of the relationship between gene structure and biological function in organisms
<b>Genotype</b>	The genetic identity or composition of an individual organism.
<b>Hazard</b>	The term hazard is associated with the potential of an agent or situation to cause an adverse effect(s)/event(s). Hazard refers to the inherent property of that agent or situation
<b>Histocompatibility</b>	The degree of similarity between organs or tissues of one individual and another so that a graft of an organ or of cells is not rejected. This compatibility depends on particular genetic similarities between the individuals involved.
<b>Imprinting</b>	Imprinting refers to chemical marks on the DNA from the dam and sire so that only one copy of a gene (either the maternal or paternal gene) is activated. The chemical mark on the DNA is usually methylation and imprinting is a form of epigenetic inheritance.
<b>Inbreeding</b>	The mating of closely related individuals, especially over many generations. Inbreeding can increase the rate of genetic improvement in farm animals but can also increase the risk of genetic defects.
<b>Integrative physiology</b>	Integrative or organismal physiology is a discipline that seeks to bring together all that is known about an animal's function to create an integrated picture of how that animal operates in its environment.
<b>In vitro</b>	In an artificial environment such as in a test tube rather than inside a living organism.
<b>Knock-in</b>	Refers to a process in which the genome of an organism is altered by replacing one particular gene sequence with another gene sequence from an external source.
<b>Knock-out</b>	Refers to a process in which the genome of an organism is altered by deleting or inactivating one or both copies of a specific gene.
<b>Lentivirus</b>	A subgroup of the retrovirus family that includes human immunodeficiency virus. Lentiviral infections are characterized by long periods of clinical latency after infection. The retrovirus family has RNA as their genetic material which translates into DNA in host cells.
<b>Marker assisted selection</b>	The use of genetic markers for selection of a linked characteristic, or trait.
<b>Mitochondrion</b>	An organelle within a cell that generates most of the cell's energy. Its DNA also maintains and expresses genetic information.

<b>Mutation</b>	A permanent transmissible change in the genetic code. It can be an insertion or deletion of genetic information, or an alteration in the original genetic information. Mutations can be caused by many factors including environmental insults such as radiation and mutagenic chemicals.
<b>Natural selection</b>	The concept developed by Charles Darwin that genes which produce characteristics that are more favourable in a particular environment will be more abundant in the next generation.
<b>Nucleotide</b>	An elementary building block of the nucleic acids DNA and RNA. Nucleotides provide the basis of the genetic code. They are adenosine, guanosine, cytosine and thymidine.
<b>Nucleus</b>	The membrane bound organelle in a cell of an animal or plant (eukaryotic organisms) that contains the chromosomes and related material.
<b>Organelle</b>	An organised structure within a cell with a specific function: e.g. a mitochondrion or chloroplast.
<b>Pathogenesis</b>	The development of disease: specifically, the cellular events and reactions and mechanisms occurring in the development of disease.
<b>Polygenic</b>	Of or relating to an inheritable character that is controlled by several genes at once; determined by polygenes.
<b>Population bottleneck</b>	Pertains to situation in which the number of parents in a population becomes very small for one or more generations and leads to a population that differs from the population existing before the bottleneck.
<b>Proteomics</b>	The study of all the proteins that are expressed in a cell,
<b>Provirus</b>	A virus that integrated into the host cell chromosome and is transmitted from one cell generation to another.
<b>Risk</b>	Risk is a function of the probability (likelihood) and severity of an adverse effect/event occurring to man or the environment [animals included here!] following exposure, under defined conditions, to a hazard
<b>Risk analysis</b>	A term with several nuances of meaning. In the current document, it refers to the Codex Alimentarius model which includes three major activities: risk communication, risk assessment and risk management.
<b>Risk assessment</b>	Refers to the process of evaluation, including the identification of the attendant uncertainties, of the likelihood and severity of an adverse effect(s) / event(s) occurring to man or the environment or animals following exposure under defined conditions to a risk source(s). A risk assessment comprises four steps: hazard identification, hazard characterisation, exposure assessment, and risk characterisation.

<b>Risk communication</b>	Refers to the interactive exchange of information and opinions throughout the risk analysis process concerning risk. It should involve not only risk assessors and risk managers, but also consumers and a wide range of other actual or potential stakeholders.
<b>Risk equity</b>	Refers to the fair treatment of those who benefit from or undertake activities that lead to risk and those who bear the consequences of risk.
<b>Risk management</b>	Refers to the process of weighing policy alternatives in the light of the result of a risk assessment(s) and of other relevant evaluations, and, if required, of selecting and implementing appropriate control options (including, where appropriate monitoring and surveillance activities).
<b>Quantitative trait loci</b>	A combination of genes that often controls economically significant genetic traits, such milk quality and quantity in dairy cattle.
<b>Re-programme/reprogramming</b>	Refers to the process whereby the DNA in body cells which is programmed or adapted to express the particular characteristics of the differentiated tissue from which it comes (e.g. muscle or nervous tissue) is de-differentiated or re-adapted to have all the capacities and potentials for developing an individual organism (i.e. becomes totipotential).
<b>RNA</b>	Ribonucleic acid, a chemical cousin of DNA that is responsible for translating the genetic code of DNA into proteins.
<b>Selective breeding</b>	See artificial selection.
<b>Sex-linked</b>	Pertains to genes carried on sex chromosomes and showing different patterns of inheritance and expression in males and females.
<b>Somatic</b>	Refers to all cells in animal apart from germ cells.
<b>Somatic cell nuclear transfer (SCNT)</b>	A process whereby the nucleus of a somatic cell is removed and placed into an enucleated oocyte; that is, an egg cell that has had its own nucleus and genetic information removed. When SCNT is used to reproduce animals it is referred to as reproductive cloning. It produces genetic near copies of the somatic cell donor rather than genetic replicas.
<b>Spermatogonia</b>	Cells in the reproductive tissues of males that give rise to spermatozoa.
<b>Telomere</b>	A repeated DNA sequence that is located at the ends of chromosomes. Telomeres shorten upon each round of cell replication.
<b>Transfect/transfection</b>	The introduction of a foreign gene (DNA) into a cell's genome.
<b>Transgene/transgenic</b>	Used in relation to organisms which have foreign genes (transgenes) incorporated into their genomes.
<b>Vector</b>	A modified virus or DNA molecule that allows transmission of new DNA into a cell.

## SUMMARY OF CONCLUSIONS

### *The Australian Animal Welfare Strategy*

1. Australian Animal Welfare Strategy provides a nationally accepted context for making public policy decisions about the health and welfare aspects of B-D animals. The AAWS sets out an inclusive approach to ensure that the interests and concerns of all interested parties are considered. These parties include the public at large, the biotechnology industry and the agricultural industries which depend upon robust and adaptable animals maintained in good health and welfare.

### *An Australian review of the health and welfare aspects of biotechnology-derived (B-D) animals*

2. There are several pre-existing and formally accredited reviews of the animal health and welfare aspects of biotechnology-derived (B-D) animals. These come from the USA, the UK and Canada. Accordingly, Australia need not start de novo and undertake a similar public review. It may be necessary, however, to extract the key points from these other reviews and test whether (1) they are appropriate for Australia, (2) there are gaps in their coverage and (3) they have been referred to in policy processes already in train.

### *The possible role of B-D animals in Australian agriculture*

3. B-D animals have demonstrated their value in biomedical research; for example, in investigating prion diseases. They may also have application in producing particular proteins for the pharmaceutical industry. However, most current reviews are sceptical about the prospects for B-D animals in agriculture. Realistic prospects for the use of B-D animals in agriculture can be gauged if a reasoned and plausible case for feasibility in the face of known basic problems is presented and if a cogent process for accrediting the health of B-D animals can be followed.
4. An exercise in foresighting following the example set by the USDA Advisory Committee on Biotechnology and 21<sup>st</sup> Century Agriculture could be useful for exploring the possible role of B-D animals within the context of Australia.
5. The inaccurate but accepted application of the term cloning to somatic cell nuclear transfer (SCNT) should be borne in mind when assessing the possible role of B-D animals in Australian agriculture. Animals cloned by SCNT are genetic near copies not genetic replicas of the animal donating the somatic cell. The gene sequence may be the same but differences occur in gene expression. Both gene sequence and gene expression are “genetic”. Accordingly, claims about the possibility for duplicating a beloved pet, the performance of a champion thoroughbred horse or the performance of elite production animals can be regarded as dubious.

### *Evaluation of the health and fitness of B-D animals*

6. Accreditation of the health and welfare of B-D animals is required as re-assurance to the general public, to provide intelligible points of reference for the biotechnology industry and to protect Australian agriculture against a handicap of

B-D animals that either have health problems or are ill-fitted to their production environment.

7. A fusion of the definition of health of the World Health Organization and the definition of animal welfare as the state of an individual as regards its attempts to cope with its environment can be used to frame assessments of the health and welfare of B-D animals. This fusion will facilitate the wealth of relevant knowledge found within comparative medicine, comparative pathology and integrative physiology. It also covers the need for an appropriate match between animals and their environments (the genotype-environment interaction). A key question is: Do appraisals of the health of B-D animals undertaken within laboratories and at research sites apply validly to every environment in which animal production may occur?
8. Genetic diversity within populations of animals is a significant biological contributor to their present and future health. Accordingly, an epidemiological or population focus may be called for when the health and welfare of B-D animals is being assessed.
9. A list of genetic hazards may be useful for establishing pathogenesis as a reference point for the surveillance and monitoring of health and welfare in B-D animals. Of necessity, this surveillance and monitoring must be based on observed functional and structural changes and on provoked behavioural and physiological responses. Lifetime fitness of B-D animals is a consideration and an innovative total diagnostic framework is required. Guidance here is available from methods used to assess the health and wellbeing of laboratory mice

#### *Scientific research*

10. It is unfortunate that the prospect of imminent application to agriculture appears to have driven research and development into B-D animals in Australia. Research should continue for its value in clarifying biological questions that may contribute more to agriculture than B-D animals themselves. Additionally, there may be some scope for B-D animals in pharmaceutical production. A multidisciplinary research approach will be valuable. An innovation could be more emphasis on organismal or integrative physiology combined with behavioural science which will clarify interactions among body systems; for example, among the nervous, endocrine and immune systems.

#### *Risk analysis*

11. The process of risk analysis presents itself as a practical method for managing the animal welfare aspects of B-D animals within the setting of the Australian Animal Welfare Strategy. Risk analysis can provide appropriate procedures for helping to reconcile objective facts and concepts about animals with the more subjective values people place on animals. It can specify the steps necessary to monitor and prevent adverse effects in B-D animals and thus allow the possible benefits of biotechnology.
12. In particular, the risk analysis procedures developed by the Codex Alimentarius Commission (CAC) could be adapted for application to animal health and welfare. CAC risk analysis includes the flexibility to be either specific or generic

depending upon need and some guiding principles that could be re-worded for animal health and welfare. An addition may be use of the “release assessment” to help decide when B-D animals can move from the research and development phase and into general use. Release assessment for B-D animals has been described by the Canadian Food Inspection Agency. The issue of risk equity is likely to be important to allow for the success of the biotechnology industry and to protect Australian agriculture against the contingency that B-D animals may have health problems or are ill-fitted to their production environment.

13. As for the viral vectors sometimes used as carriers of foreign DNA in transgenesis, risk analysis could be applied to the question of whether these will be hazards. This use could prevent one of two harms: either the rejection of beneficial technological advances or the acceptance of poorly characterised risks.
14. A review of the Office of the Gene Technology Regulator (OGTR) and its covering legislation is in train. Information about this review (terms of reference etc.) can be found at <http://www.tga.gov.au/gene/gtreview.htm>. Risk analyses are routinely performed by the OGTR. NCCAW could provide advice and assistance on the nature of the risk analysis required for animal welfare under the umbrella of the Australian Animal Welfare Strategy (AAWS). The AAWS seeks to ensure formal processes for community involvement in the development and implementation of welfare standards.

## GENERAL SUMMARY

This advice note deals with the health and welfare consequences that may accompany the application of biotechnology, genetic modification and cloning to animals, particularly farm animals. It arises from a paper that was considered at the 36<sup>th</sup> meeting of the National Consultative Committee on Animal Welfare (NCCAW) held in Canberra 8–9 September 2005. NCCAW 36 requested that the paper be edited in line with comments from members and be placed on the NCCAW website.

The advice note begins by outlining (1) other reviews of the health and welfare of B-D animals undertaken in Australia elsewhere in the world, (2) the scope of biotechnology, genetic modification and cloning that applies to animals, and (3) the prospects for biotechnology derived (B-D) animals in practical agriculture. It then considers the genetic hazards associated with the established breeding technologies (artificial insemination etc), genetic engineering and cloning. These hazards have been assembled to provide a frame of reference that may be useful when the appropriate scientific disciplines are applied to the assessment of the health and welfare of B-D animals. The disciplines in mind are (1) integrated physiology and behavioural science and (2) comparative medicine and pathology.

The advice note also describes risk analysis and how the process that has been designed for food safety could be adapted for use with animal health and welfare, particularly within the context of the Australian Animal Welfare Strategy. Here it could assist in clarifying the complex of ethical, cultural, social, scientific and economic issues associated with B-D animals and thus facilitate evidence-based policy.

Conclusions are that:

1. The Australian Animal Welfare Strategy is a nationally accepted context for making policy decisions about the welfare aspects of B-D animals;
2. Australia can heed the several pre-existing and formally accredited public reviews of the animal health and welfare aspects of B-D animals that have come from the UK, USA and Canada and need not undertake a similar review;
3. Most current reviews are sceptical about the prospects for B-D animals in agriculture but realistic prospects can be gauged if a reasoned and plausible case for feasibility in the face of known problems and a cogent process for accrediting the health of B-D animals is presented. Scenario analysis could be useful.
4. The accreditation of the health and welfare of B-D animals is required to reassure the general public and to ensure that Australian agriculture is not burdened with animals with health problems or that are ill-fitted to their environment. Health assessment should be situation specific and include the fit between animals and their environment, an epidemiological approach, lifetime fitness and innovative diagnostic packages that heed integrative physiology and behavioural science and look at provoked physiological and behavioural responses.
5. Scientific research into B-D animals should continue. It may clarify some biological questions with greater value to animal agriculture than B-D animals themselves. A multidisciplinary approach is regarded as valuable and should extend to organismal or integrative physiology and behavioural science.

6. The process of risk analysis, exemplified by that developed by the Codex Alimentarius Commission for food safety, presents itself as a practical method for managing the animal welfare aspects of B-D animals under the Australian Animal Welfare Strategy and for reconciling objective facts and concepts about animals with the more subjective values people place on animals. Risk equity is a consideration and seeks to balance the interests of those who are responsible for creating risk and those who may bear the consequences of risk. Risk analysis could be applied to the question of whether the viral vectors sometimes used as carriers of foreign DNA in transgenesis will be hazards. The aim would be to prevent one of two harms: either the rejection of beneficial technological advances or the acceptance of a poorly characterised risks.
7. NCCAW could be a source advice and assistance to the Office of the Gene Technology Regulator on risk analysis for the health and welfare of B-D animals and in light of the Australian Animal Welfare Strategy. In addition, NCCAW provides opportunities for consultative processes.

## 1. INTRODUCTION

The present advice note assembles and analyses information about the animal welfare consequences that may arise from the assortment of procedures within the compass of biotechnology, genetic modification and cloning and which occur in so-called biotechnology-derived (B-D) animals (terminology of the Canadian Food Inspection Agency, CFIA). Its aim is to provide accessible and reliable knowledge to facilitate discussion within the contemporary context of the Australian Animal Welfare Strategy (AAWS, 2004), see Conclusion 1. Among other things, the AAWS establishes science as an important ingredient in decision-making about animal welfare. *“Australia recognises the essential role of science in animal welfare, to provide evidence and concepts that support value based decisions, as a foundation for animal welfare standards and innovation and as a bridge to ‘best-practice’ animal husbandry”*. The technologies that apply to animal breeding are the present focus. Health protection technologies such as vaccination and selection for disease resistance involve an additional set of issues and require separate consideration.

A secondary, but equally important, purpose of the advice note is to float the possibility of applying the processes of risk analysis to the activities of AAWS. Risk analysis provides a structured and manageable approach for bringing science to bear on health and environmental risks and for facilitating communication among policy makers, the “risk-interested” public, risk professionals and scientists (FAO/WHO, 1997; European Commission, 2000 and 2003; Presidential/Congressional Commission on Risk Assessment and Risk Management, 1997). Pertinently, risk analysis has been canvassed for a similar use in public policy about animal welfare (European Commission, 2002) and, in particular, the animal welfare impacts of transgenesis, biotechnology and cloning (Van Reenen et al., 2001; Moreau and Jordan, 2005).

The present advice note is not intended to be risk assessment of the sort required under the formal risk analysis process. Rather, its purpose is to outline a set of genetic considerations that may provide a useful frame of reference for exploring issues and applying the body of scientific knowledge to the animal welfare aspects of biotechnology, genetic modification and cloning. Farm animals are the focus in the

present paper but the genetic considerations may also apply to other classes of animals. The underlying thesis is that every breeding technology, including age-old selective breeding, can cause harm to individual animals and populations of animals through an identifiable set of defects and deficiencies in genes and their expression. These identifiable defects and deficiencies in genes and their expression constitute genetic “hazards” in the parlance of risk analysis. Genetic hazards include changes within the genetic code (DNA) itself and epigenetic modifications that lead to changes in gene expression. The term, epigenesis, refers to ‘alterations in DNA function without alterations in DNA sequence’ (Jones and Takai, 2001). The present advice note assembles a set of genetic hazards from a stepwise examination of each of the breeding and genetic technologies that apply to animals.

## **2. PRELIMINARY CONSIDERATIONS**

### **2.1 Other reviews of biotechnology, genetic modification, cloning and animal welfare**

In 2000 and 2002, Lewis et al. provided a viewpoint on the use and limitations to use of cloning and transgenesis in farm animals in Australia. They concluded that it would be many years before produce from cloned or transgenic animals would enter the human food chain. They recognised the need for appropriate government guidelines and that consumers would have the final say. They also pushed for a continuing research effort in Australia to foreclose on a future possibility that Australian farmers may need to buy the technology from overseas on a seller's market. A more recent viewpoint, expressing similar sentiments, comes from Seamark in 2003.

Several comprehensive reviews of the impacts of biotechnology, genetic modification and cloning on animal health and welfare and their public policy ramifications are available from public bodies in the United Kingdom and the USA. These public bodies include the US National Research Council (2002a, 2002b and 2004), the Farm Animal Welfare Council (FAWC) of the UK (1998 and 2004) and the Royal Society of the UK (2001). The Pew Initiative of Food and Agriculture of the Pew Charitable Trusts has also published the proceedings of a public conference in 2003 on animal cloning and the human food chain.

The 1998 report of the UK Farm Animal Welfare Council (FAWC) on the implications of cloning for the welfare of animals contains a set of recommendations that were responded to by the UK Department of Agriculture Fisheries and Forestry (DEFRA) in 2003 is also shown. It is noteworthy that DEFRA does not regard the use of cloned animals in agriculture as imminent and requiring special public policies; an opinion echoed in the DEFRA response in 2003. Excerpts from FAWC recommendations and the DEFRA response that illustrate this viewpoint are shown in Box 1.

#### **Box 1 – Excerpts from UK DEFRA’s response to the 1998 Farm Animal Welfare Council report on the use of cloned animals in UK agriculture.**

**FAWC recommendation 3:** On the need for research into the health consequences of cloning

*DEFRA are not supporting research into this as cloned farm livestock are not considered to be of relevance to the future of sustainable livestock farming in the UK.*

**FAWC recommendation 4:** On the need for a moratorium on the use of cloned animals until health problems are resolved

*DEFRA: There are no plans to clone farm animals commercially. We, therefore, do not agree with the need for an immediate moratorium on the commercial use of cloning by nuclear transfer. We will keep the situation under review as the technology advances.*

**FAWC recommendation 7:** A further aspect of good welfare lies in controlling the competence of those who carry out procedures.....the Royal College of Veterinary Surgeons should be consulted to explore the feasibility of any of the procedures involved in cloning by nuclear transfer which are "acts of veterinary surgery".

*DEFRA: As the commercial cloning of farmed animals is still a long way off, we do not have to take immediate action.*

The most recent report by the UK Farm Animal Welfare Council on animal welfare and the breeding technologies for farm animals (2004) suggests a watching brief on developments in genetic modification and cloning. Relevant paragraphs are:

95. The extent to which genetic modification will become incorporated into future livestock breeding strategies may well be determined, not by scientific developments, but by public acceptability of the technology. Opposition to GM crops by consumers, retailers and environmentalists continues to influence the commercial application of GM Technology in the plant sector, and there is no reason to believe that a similar level of opposition would not develop if the technology became incorporated into livestock breeding. Given the above, and also the rapid pace of developments in this area, FAWC recognised the need to remain informed regarding this issue.
103. At the present time, within the UK, all cloning work including any work on possible commercial applications, is confined to research establishments and is done under the protection of A(SP)A<sup>1</sup>. However, in the light of predictions made by some commercial breeding companies involved in cloning work, it is essential that FAWC keep a close watching brief on developments in this field.

The advantage of access to pre-existing and formally accredited reviews implies that Australia need not start from scratch and undertake a similar public review, except to ensure full and inclusive consultation. It may be necessary, however, to extract the key points from these other reviews and test whether (1) they are appropriate for Australia, (2) there are gaps in their coverage and (3) they have been referred to in policy processes already in train (see Conclusion 2).

## **2.2 Evaluation of animal welfare**

For present purposes, animal welfare is addressed through a fusion of two ideas. The first idea is the concept of health that applies to people and which is described in the 1946 Constitution of the World Health Organization: *Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity*. This depiction of "health" embraces most but not all biological aspects of animal welfare. It may facilitate the application of the wealth of relevant knowledge found within comparative pathology and medicine. The second idea is the definition of animal welfare as the state of an individual as regards its attempts to cope with its environment (Broom, 1986). This definition may facilitate the application of ideas found within integrative physiology: the discipline that seeks to bring together all that is known about an animal's function to create an integrated picture of how that animal operates in its environment.

A health-based approach covers risks to the instrumental value of animals that arise from biotechnology, genetic modification and cloning. It also goes some way towards

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<sup>1</sup> UK Animals Scientific Procedures Act 1986.

clarifying similar risks to the intrinsic value of animals. In this regard, instrumental value refers to the usefulness animals have for people and intrinsic value refers to the value animals have in their own right. Instrumental and intrinsic values overlap when the issue is maintenance of genetic diversity within populations of animals and benefit to future generations of both people and animals is in mind.

The WHO definition of health issues is silent on a basic biological concept that has major implications for the health and integrity of animals. It is the need for an appropriate match between the genetic makeup and animals and their environment. This concept is at the core of Donald Broom's 1986 definition of animal welfare. Genotype-environment interactions and the nature-nurture issue relevant. The point is that disturbance to the genetic makeup of biotechnology-derived (B-D) animals may necessitate compensatory changes in their nutritional, physical and social environments. Since genetic diversity within populations of animals is a significant biological contributor to their present and future health, an epidemiological or population focus may be called for when the health and welfare of biotechnology-derived (B-D) animals is being assessed (see Conclusion 8).

### **2.3 The scope of biotechnology, genetic modification and cloning**

The following definitions come from a paper by Moreau and Jordan (2005) and the Advisory Committee on Biotechnology and 21<sup>st</sup> Century Agriculture of the USDA. They may help delineate the issues covered by "biotechnology, genetic modification and cloning".

#### **2.3.1 *Biotechnology***

According to the USDA Advisory Committee on Biotechnology and 21<sup>st</sup> Century Agriculture (2005), biotechnology is a range of tools, including traditional breeding techniques, that: (1) alter living organisms (or parts of organisms) to make or modify products; (2) improve plants or animals; or (3) develop micro-organisms for specific uses. Discussion on biotechnology frequently dwells on "products of modern biotechnology and transgenic (or genetically engineered) organisms (or their products), namely organisms produced through genetic engineering or recombinant DNA processes, and the products derived from them".

The United Nations Convention on Biological Diversity (1993) refers to biotechnology as any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.

Article 3 of the Cartagena protocol on biosafety (2000), a supplementary agreement proposed under the United Nations Convention on Biological Diversity (see Sendashonga, Hill and Petrini, 2005), delineates biotechnology as: "The application of: (a) *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or (b) fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding or selection".

### 2.3.2 *Biotechnology-derived animals*

The term “biotechnology-derived animals” (B-D) comes from the Canadian Food Inspection Agency; see Moreau and Jordan (2005). The term “may include but is not limited to the following categories of animals:

- genetically-engineered or modified animals, in which genetic material has been added, deleted, silenced or altered to influence the expression of genes or traits;
- so-called cloned animals derived by nuclear transfer from embryonic or somatic<sup>2</sup> cells;
- chimeric animals [single animals produced from genetically distinct cells derived from two different fertilised eggs];
- interspecies hybrids; and
- animals derived from in vitro cultivation, such as oocyte maturation or manipulation of embryos.”

## 2.4 **Prospects for biotechnology, genetic modification and cloning**

Biotechnology-derived (B-D) animals have demonstrated value in biomedical research (see Niemann and Kues, 2003; Wheeler et al, 2003). One topical example relates to research on prions where B-D animals have uniquely facilitated an understanding of the epidemic of BSE in cattle and the associated epidemic of variant Creutzfeldt-Jakob disease in people. B-D animals may also be useful in the production of particular proteins for the pharmaceutical industry.

There are two views on the imminent appearance and practicability of biotechnology-derived animals in commercial agriculture. These views may also apply to biotechnology-derived companion and zoo animals. One is a guarded view. The other is an upbeat view. The guarded view is exemplified in reports of the Food Safety Department of the WHO (2005) and the Royal Society (2001), in a “policy forum” paper of Gordon (1999) and, more recently, in a review by Mapletoft and Hasler (2005). The WHO report states:

“Food derived from GM livestock and poultry are far from commercial use. Several growth-enhancing novel genes have been introduced into pigs that have also affected the quality of the meat, i.e. the meat is more lean and tender. This research was initiated over a decade ago but owing to some morphological and physiological defects developed by pigs, these have not been commercialized.

Many modifications to milk have been proposed that add either new proteins to milk or manipulate endogenous proteins. Recently, researchers in New Zealand developed GM cows that produce milk with increased levels of casein protein. Use of such protein-rich milk would increase the efficiency of cheese production. Other work aims to reduce the lactose-content of milk, with the intent of making milk available to the population of milk-intolerant individuals.

Other applications of genetic modification in animal production in the early stages of research and development include improvement in disease-resistance, increased birth rates in sheep, altered sex ratio in poultry, increased egg production in poultry by creating two active ovaries, and improved feed conversion in the “enviropig” (environmentally friendly pigs that excrete less phosphorus). Most of this work is still theoretical and therefore estimates of time frames for possible commercial introductions of any of them are unavailable.

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<sup>2</sup> Somatic cells are cells taken from body tissues such as skin, mammary gland and liver and not from the reproductive tissue of ovaries and testes.

The Royal Society (2001) sums up progress towards genetically-modified farm animals as follows:

Despite the growing list of GM animals in agriculture, transgenically derived animal food products are still a long way off<sup>3</sup>. No GM livestock for food production are close to being evaluated by regulatory authorities in the UK. Difficulties arise for several reasons.

- The efficiency of genetic modification of the farm animal genome is low (less than 1% of GM offspring in pigs, sheep, goats and cattle).
- Current methodologies use approaches that have resulted in high levels of embryonic loss or damage.
- The longer breeding cycles of farm animals and low number of offspring (except pigs) and high financial production costs limit the pace at which GM livestock can be created.
- Detailed knowledge of the farm animal genomes is still incomplete, as are the functions of their genes.
- This applies in particular to understanding the genetic factors controlling production traits; control of normal tissue and organ-specific gene expression; control of transgene expression; the lack of replication of defective retroviral vectors for gene transfer and ways of improving transgene constructs.
- Many of the desirable traits such as disease resistance and production traits are polygenic and require the alteration and coordinated expression of several genes, many of which have yet to be defined.
- Funding agencies are not supporting GM livestock projects to a high level and returns for venture capital are regarded as low.
- Concerns about animal welfare and food safety aspects of food animal biotechnology are justified.

According to Mapletoft and Hasler (2005), cloning of cattle through somatic cell nuclear transfer and production of cloned, transgenic cattle has been demonstrated but the technology is unpredictable, expensive and inefficient and is accompanied by animal wastage. The technology may have prospects for the pharmaceutical industry but is expensive and inefficient and the “benefits in agriculture are likely to be minimal in the near future”. Ian Wilmut, the scientist involved in the production of “Dolly”, the first cloned sheep, co-authored a paper which concluded that reproductive cloning required considerable improvements in efficiency before it could have wide application to livestock (Paterson et al., 2003).

In contrast to this multitude of guarded views, Niemann et al. (2005) are broadly optimistic about biotechnology-derived animals in agriculture. “The authors anticipate that within the next five to seven years genetically modified animals will play a significant role in the biomedical arena, in particular via the production of valuable pharmaceutical proteins and the supply of xenografts (Table II). Agricultural applications are already being prepared (39), but general public acceptance may take as long as ten years to achieve. As the complete genomic sequences of all farm animals become available, it will be possible to refine targeted genetic modification in animal breeding and to develop strategies to cope with future challenges in global agricultural production.”

Rather than attempting to foretell whether the upbeat or more guarded view about the use of B-D animals in agriculture will prevail, the USDA Advisory Committee on Biotechnology and 21<sup>st</sup> Century Agriculture (2005) undertook an exercise in scenario planning. Accordingly, a set of different futures characterised by various degrees of success for biotechnology was explored. The purpose was not to predict or endorse a

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<sup>3</sup> The reference provided in the report from the Royal Society is a “personal communication”. No list of all current endeavours to produce biotechnology-derived animals appears to be available in the public domain.

given scenario but to understand the “implications of differing outcomes”. Three scenarios were created. They were called “Rosy Future” (where biotechnology exceeded expectations), “Continental Islands” (where international harmonisation of regulation did not occur) and “Biotech goes niche” (where the big claims of biotechnology were not be realised but some small biotechnological applications came to fruition).

An exercise in foresighting or scenario analysis following the example set by the USDA Advisory Committee on Biotechnology and 21<sup>st</sup> Century Agriculture could be useful for exploring the possible role of biotechnology-derived animals within the context of Australian agriculture (see Conclusion 4). In this regard, a study undertaken in 2003 by the Genesis Faraday group (a partnership between Government and Industry in the UK) provides an outsider's view of the status of research into genetics and genomics<sup>4</sup> of sheep and cattle in Australia and New Zealand. Cloning and transgenic technology are hardly mentioned in the Genesis Faraday study. Genetic markers<sup>5</sup> and the identification of quantitative trait loci<sup>3</sup> (QTL), as aids in the selection of individual animals for breeding programs, tend to dominate the research described.

The impediments to progress towards the use of B-D animals in agriculture, particularly transgenic animals, have been identified (see Gordon, 1999)<sup>6</sup> and will be expanded later in the paper. An understanding of these impediments helps to clarify prospects for the use of B-D animals in practical farming situations. In short, the impediments to progress for transgenesis relate to uncertainties in the process that can lead to a garbling of genetic code of transgenic animals. These disruptions or “garblings” in the genetic code can occur in the following ways:

- *DNA transferred via transgenes integrates at random into the DNA of the host.* It is known that genes must be positioned appropriately, that is in the appropriate place in the appropriate chromosome, for their proper expression and the normal function of whole animals.
- *The number of gene copies inserted into the DNA of the host cannot be controlled.* Possession of more than one gene copy in the genome can lead to overproduction of the gene product and interference with normal function of whole animals.
- *Transfer of DNA may lead to significant rearrangements of host genetic material.* Again, it is known that the genes must be positioned appropriately for their proper expression and normal function of whole animals. The transgene may be interposed between genes that must be next to one another for their normal expression.

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<sup>4</sup>Genomics: the study of the relationship between gene structure and biological function in organisms. Other neologisms related to biotechnology are proteomics, which is the study of all the proteins that are expressed in a cell, and informatics (bioinformatics), which is the science of managing and analyzing biological data using advanced computing techniques.

<sup>5</sup>Marker Assisted Selection: Use of genetic markers for selection of a linked characteristic, trait, or disease associated gene. Quantitative trait loci: A combination of genes that often controls economically significant genetic traits, such as disease resistance in animals and, in dairy cattle, milk quality and quantity.

<sup>6</sup>Jon Gordon and Frank Ruddle demonstrated the possibility of transgenesis in 1981 when they transferred a human gene to a mouse.

- “*Insertional mutagenesis*” may occur. The problem here is that the transgene may be inserted into the DNA sequence of a pre-existing gene and thus change or mutate this gene.

Targeted gene transfer, where genes are inserted into a predetermined site in the genome may assist in rectifying the problems listed above.

The impediments to progress for somatic cell nuclear transfer (cloning) relate to health problems observed in cloned animals which have their basis in disrupted gene expression. These health problems are:

- an unacceptable death rate in surrogate dams;
- a low birth rate;
- a high rate of loss in late pregnancy;
- congenital abnormalities;
- death of neonates;
- an unacceptable death rate after the neonatal period; and
- poor production performance in dairy cattle.

To conclude, realistic prospects for the use of B-D animals in agriculture can be gauged if a reasoned and plausible case for feasibility in the face of known basic problems is presented and if a cogent process for accrediting the health of B-D animals can be followed (see Conclusion 3). The establishment of a National Standing Committee to oversee the development of cloning technology remains a possibility in the UK (FAWC, 2004).

## 2.5 Risks versus hazards

A synopsis of risk analysis is presented later in this paper. The distinction between “risk” and “hazard” helps frame discussion of the animal welfare impacts caused by biotechnology, genetic modification and cloning. These technologies can be considered elementary hazards with a capacity for generating one or more resultant hazards within the genetic material of biotechnology-derived (B-D) animals (“genetic hazards”). Box 2 contains definitions from the Scientific Steering Committee SSC of the European Commission (2000) plus some additions from the Canadian Food Inspection Agency (CFIA) (2005), from Moreau and Jordan (2005).

### Box 2: Hazards and Risks

#### **Hazards**

SSC: The term **hazard** is associated with the potential of an agent or situation to cause an adverse effect(s)/event(s). **Hazard** refers to the inherent property of that agent or situation.

CFIA: A **hazard** is a source of risk that does not necessarily produce risk (i.e. a source with the potential to produce risk). A **hazard** produces risk only if an exposure pathway exists and if exposure creates the possibility of adverse consequences.

## **Risks**

SSC: **Risk** is a function of the probability (likelihood) and severity of an adverse effect/event occurring to man or the environment [animals included here!] following exposure, under defined conditions, to a hazard.

CFIA: Objective measurement and scientific repeatability are key features of **risk** evaluation. **Risk** differs from “likelihood” because it embraces the severity of possible consequences as well as the probability of their occurrence.

### **3. ESTABLISHED BREEDING TECHNOLOGIES**

The established breeding technologies are selective breeding (particularly where the selection pressure has been intensified) and the assisted breeding technologies of (1) artificial insemination, (2) superovulation and synchronisation of oestrus, (3) embryo transfer, (4) the freezing of semen and embryos, (5) ultrasound scanning for pregnancy, (6) in vitro fertilisation, (7) semen and embryo sexing and (8) cloning by means of embryo splitting.

#### **3.1 Selective breeding**

Domestic animals have been changed genetically and breeds of domestic animals have been fixed by the selection and controlled mating of individual animals with characteristics regarded as desirable. The process involved is artificial selection, a term coined by Charles Darwin to make a contrast with natural selection, which is essential to the theory of evolution (Darwin, 1872).

The pace of genetic change and the fixing of livestock breeds accelerated during the industrial revolution with its leaping advances in agriculture. Selective breeding has continued to intensify with help from technologies such as the objective measurement of performance and artificial insemination. Once marker-assisted selection becomes available, selection pressure will intensify.

Animals with improved production traits form the basis of modern animal-based agriculture and make a major contribution to the human food supply.

##### *3.1.1 Genetic hazards associated with selective breeding*

Selective breeding for improved production performance may have unintended genetic consequences. These are:

- An increased incidence of genetic disorders that can result either from the increased frequency of pre-existing faulty genes through *genetic drift* or, more seldom, through the perpetuation of *injurious mutations* in genes;
- The appearance of inbreeding depression, which has an insidious onset and vague signs such as decreases in reproductive performance or increases in disease susceptibility.

##### Single-gene disorders, chromosome aberrations and polygenic disorders

Genetic drift and pre-existing faulty genes provide the most important pathway for breeding abnormalities, genetic disorders and the “inborn errors of metabolism”. Genetic drift refers to changes in gene frequencies brought about by chance. Genetic

drift becomes larger and more significant as the breeding population becomes smaller. Genetic drift is responsible for the *founder effect* in which a small number of parents initiate a new population and results in a *population bottleneck*. The *founder effect* becomes significant for genetic disease when a small founding population carries injurious genes. Significantly, the *founder effect* will not lead to genetic disease if injurious genes<sup>7</sup> are not present in the founding population.

Genetic disorders can result from single-factor Mendelian inheritance where the cause is a single gene; which may be dominant (always expressed), recessive (only expressed when both copies of the gene are recessive) or sex-linked (where the gene is on a sex chromosome and is only expressed in either males or females). Haemophilia is an example of a sex-linked single gene disorder. It has been recorded in people, cats, dogs, cattle, horses and sheep. A compilation of genetic disorders in animals is available on the Internet: Online Mendelian Inheritance in Animals (OMIA) at <http://www.angis.org.au/Databases/BIRX/omia/>. (Professor F.W. Nicholas, Sydney University)(Nicholas, 1998). A comprehensive account of the genetic science behind the diagnosis of genetic disorders is available (Nicholas, 1989).

Gross abnormalities or aberrations in chromosomes can lead to genetic disorders. A list of such abnormalities maintained at the OMIA Internet site.

Many inherited disorders run in families and result from the action of multiple genes (polygenic). Individuals in such families have a familial tendency towards, or an increased liability for, particular disorders. Congenital heart disease in people and hip dysplasia in dogs are examples of disorders where polygenic or multifactorial inheritances operate.

#### Inbreeding depression

Inbreeding refers to the extent of mating between closely related individuals. Inbreeding is used to preserve desirable or eliminate undesirable characteristics in animals. In doing so, inbreeding may increase the frequency of unfavourable as well as favourable genes. As a result, inbreeding may cause the segregation of various kinds of congenital defects and, more importantly, may lead to a general decline in fertility and viability of the inbred animals. The decrease in performance produced by inbreeding and the expressed effect of “subvital” harmful genes is inbreeding depression. Inbreeding depression is expressed as a decrease in general fitness shown by poor rates of reproduction and disease resistance. Inbreeding depression may be insidious in onset.

#### Genotype and environment interaction

In many instances, modern livestock have been selected for high production performance, which demands a correspondingly high food intake to meet requirements for protein, energy, minerals and accessory nutrients. If nutrient intakes are insufficient, the metabolic imperatives for growth, lactation or egg production may take precedence over other functions and protein, energy and mineral will be

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<sup>7</sup> Mutation in germline cells is another possibility for the appearance of injurious genes. According to genetic theory, non-recurrent mutations are of little significance in changing the gene frequency of a population and are unlikely to perpetuate an injurious gene. In contrast, recurrent mutation in genes with an intrinsically high rate of mutation is capable of changing the gene frequency in a population and could perpetuate an injurious gene.

drawn from body tissues. The consequences may be expressed in the array of so-called production diseases that occur when production in high producing strains of animals plus their energy requirements for environmental adaptation exceeds their dietary inputs.

The Farm Animal Welfare Council of the UK (2004) recommended that: “industry, possibly with Government support, should sponsor research and training programmes for the development of husbandry systems to support the demands of new genotypes in relation to their production system”. Such research may not necessarily fall under the heading of “animal welfare”. Nevertheless, it is likely to have a material impact on the health and wellbeing of animals.

Government and industry research partnerships are already in place in Australia for the animal industries. In addition, research into husbandry systems is already being undertaken. In this connection, organismal or integrative physiology (the study of the physiological function of whole organisms) has been rediscovered for its value in providing sound anchor points for research efforts at the molecular and cellular level. Research programs in integrative physiology combined with behavioural science can complete a multidisciplinary mix that will aid in the success of biotechnology (see Recommendation 10). These programs allow for an understanding of the new properties that emerge in B-D animals. Furthermore, they are required to characterise the health and welfare status of these animals (see Conclusion 9).

### **3.2 Assisted breeding technologies**

No special genetic hazards appear to be associated in farm animals with (1) artificial insemination, (2) superovulation and synchronisation of oestrus, (3) embryo transfer, (4) the freezing of semen and embryos, (5) ultrasound scanning for pregnancy, (6) in vitro fertilisation, (7) semen and embryo sexing and (8) cloning by means of embryo splitting. However all these technologies can be associated with unacceptably high rates of inbreeding; reviewed by Nicholas (1996). Furthermore, artificial insemination and embryo transfer can facilitate the transmission of infectious disease. This was recognised at the outset and comprehensive, well-executed and evidence-based disease prevention measures have been formulated for artificial breeding in livestock.

Long term studies in the mouse, including observations of the aging process, have shown that individuals derived from frozen embryos have defects in structure, function and behaviour (Dulioust et al, 1995).

## **4. BIOTECHNOLOGY APPLIED TO BREEDING**

Two broad classes of biotechnology derived (B-D) animals are considered for the genetic hazards that might apply to them. These are:

- genetically-engineered or modified animals, in which genetic material has been added, deleted (“knocked-out”), silenced or altered (knocked-in) to influence the expression of genes or traits; and,
- animals derived by nuclear transfer from embryonic or somatic cells, known colloquially but inaccurately as cloned animals.

Another technology producing B-D animals is restricted to biomedical research and is not considered here. It is random mutagenesis where mutations are induced chemically in the sperm generating cells of male mice. These male mice are then bred and the offspring are screened for physical characteristics (phenotypes) of interest.

#### **4.1 Genetically-engineered or modified animals**

The first successful introduction of engineered DNA (transgenes) into the genetic material (the genome) of mice and its subsequent transmission to progeny occurred 24 years ago (Gordon and Ruddle, 1981). The next significant step came in 1982 when Richard Palmiter and colleagues demonstrated remarkably increased growth in mice derived from fertilised eggs injected with a transgene that fused the gene for rat growth hormone with the mouse metallothionein gene. In this instance, the metallothionein gene responded to dietary zinc to cause the production of growth hormone. This fusion transgene was seen to have a potential for studying the physiology of growth hormone, for accelerating animal growth and as a disease model for gigantism. The growth hormone fusion gene was used to extend transgenic technology to pigs (Hammer et al., 1985).

Genetic engineered or modified animals are created through the process of transgenesis. In this process, a gene (a transgene) is taken from another organism and introduced deliberately, with or without other changes in its chemical structure of DNA, into the genetic material (the genome) of target animal. Transgenes are frequently constructed by attaching the gene of interest to so-called promoter-regulatory sequences of DNA. These are designed to direct the anatomical distribution of the gene of interest and also to regulate its expression.

Transgenes are “transfected” or transferred into target animals in various ways. Transgenes can be introduced into cell nuclei of target animals by microinjection or by a variety of other methods including electroporation (introduction of DNA by electrical pulses), treatment with polycations or the use of lipid vesicles. The first method adds the transgene to embryonic stem cells growing in tissue culture. These “transformed” stem cells are then injecting into the cell mass of an early embryo (the blastocyst). The second method adds the transgene to pronuclei found in eggs shortly after fertilisation. In both methods, fertilised eggs or early embryos containing the transgene are surgically implanted into foster mothers. The resulting offspring are tested to determine whether they contain the transgene. If they do, they can be used as foundation stock for breeding transgenic lines or strains of animals by traditional methods. Unless gene-targeting procedures are employed, the introduction of DNA into cells in the form of transgenes is random. As a consequence, the results are generally variable and uncertain (Gordon, 1999; Van Reenen et al., 2001; NRC, 2002a).

So-called vectors can be used to introduce transgenes into the genome of a target animal. Lentiviruses, with deletions of viral genes that are required for replication, have been used for this purpose, for example by Hofmann et al. (2003), who delivered a transgene for green fluorescent protein into pigs. Lentiviruses are a class that includes the agents of diseases such as HIV-AIDS in people, maedi-visna in sheep, infectious anaemia in horses and immunodeficiency syndromes in cattle and cats. Concerns are the possibility that evolutionary forces will change the virus vector and that the vector will be passed to offspring and disseminated within animal populations. This last concern applies particularly to farm animals in general agriculture. Hofmann’s group have unpublished results (Pfeifer et al., 2004) that show when lentiviral vectors were

incorporated into animal DNA they were passed from generation to generation as provirus.

The generation of transgenic farm animals usual requires a sequence of technical steps as follows (Van Reenen et al., 2001): (1) the collection of eggs from superovulated females, (2) the maturation of these eggs in artificial culture conditions, (3) the *in vitro* fertilisation of these eggs in preparation for transfection with the transgene, (4) *in vitro* embryo culture after transfection and (5) embryo transfer to surrogate dams. Each of these technical steps can land animals with problems.

So-called gene targeting procedures are now available that allow the precise introduction of planned mutations into selected sites in the genome, usually the mouse genome. When gene targeting is aimed at selectively and completely eliminating the activity of a gene already present in the genome, “knock-out” or “null mutant” animals are produced. The production of knockout pigs that lack the gene for alpha1,3-galactosyltransferase is one of the steps towards pig-to-human xenotransplantation. When gene targeting is aimed at introducing point mutations in genes already present in the genome, “knock-in” animals are produced.

#### 4.1.1 Genetic hazards of transgenesis

The genetic hazards associated with transgenesis result from defects and deficiencies in genes and their expression and can be attributed to (1) random integration of donor DNA into recipient DNA, (2) lack of control of the number of gene copies inserted, (3) significant rearrangements of host genetic material, and (4) a troublesome frequency of insertional mutagenesis (Gordon, 1999). Van Reenen et al. (2001) placed these genetic hazards into three broad classes: (1) insertional mutations; (2) problems with the expression of transgenes; and (3) problems derived from breeding technologies themselves. Infectious diseases hazards have been mentioned earlier when lentiviral vectors were discussed.

##### Insertional mutations

Gene transfer methods that involve microinjection, implantation of transgenic spermatogonia (male germ cells), retrovirus injection, or sperm-mediated gene transfer all result in the random integration of the transgene into the genome (Niemann et al., 2005). As a consequence, all methods will produce insertional mutations. These occur when the entry point of the transgene is within or sufficiently close to the DNA sequence of a gene already within the genome of the recipient. The transgene disrupts the chemical structure of the pre-existing gene and destroys its function. In some instances, there may be an impact on health. The frequency of insertional disruptions has ranged from 7 to 20% in transgenic mice (quoted in Van Reenen et al., 2001).

##### Problems in the expression of transgenes

The anatomical, physiological and behavioural integrity of individual animals depends upon the exquisite regulation and orchestration of gene expression. Transgenes may express their effects in transgenic animals at the wrong place, at the wrong time or at the wrong stage of an animal's development. Van Reenen et al (2001) refer to problems arising when milk protein transgenes express themselves in tissues such as heart, kidney and brain.

Sometimes, the transgene may disrupt the web of interactions between other genes. In this connection, the site of insertion of transgenes has its own consequences. There may be impacts on *epistasis* where one gene permits or masks the expression of one or more other genes. In some instances, genes may be *pleiotropic* and have multiple effects.

At other times, the product of the transgene may be in overabundance and thus tax the mechanisms involved in maintaining physiological and behavioural stability and resilience. Interactions among body systems (for example, among the nervous, endocrine and immune systems) are known to be complex and precisely regulated (McEwen et al., 1997). A transgene product may disturb important relationships. Growth hormone for example is now known to be a regulator of the immune response as well as an animal's size. The interactions between body systems are the subject-matter of integrated or organismal physiology and have important implications for human and veterinary medicine (see Conclusions 9 and 10 about the value of integrative physiology).

#### Problems arising from breeding technologies

The creation of transgenic animals involves breeding technologies (the *in vitro* fertilisation of eggs in preparation for transfection with the transgene and the *in vitro* embryo culture after transfection) that are associated with problems in their own right. These problems may appear in different combinations and can include the large offspring syndrome, a high incidence of abortion throughout pregnancy, an increased incidence of congenital malformations, an increased birth weight, a longer gestation period and a high perinatal mortality rate (Van Reenen et al., 2001; Lazzari et al., 2002; Walmsley et al., 2004; Farin et al., 2004). The most common explanation is pitched broadly at some sort of interference with the expression of developmentally important genes; i.e. an epigenetic effect.

#### **4.2 Nuclear transfer (cloning)**

A major advance towards the technology popularly known as cloning occurred in 1951 when Briggs and King reported that normal hatched tadpoles could be obtained by transferring the nucleus of an early embryonic cell into an enucleated egg of the same frog species (Gurdon and Byrne, 2003). This finding answered an important question in biology. It demonstrated that genes not required after embryonic cells differentiate into specialised tissue cells are retained and are not either discarded or permanently switched off.

Mammalian eggs are much smaller than those of amphibians and it was not until 1983 that live mammals (mice) were produced when the nucleus from a germ cell (a fertilised egg) was inserted into an enucleated egg (McGrath and Solter, 1983). In 1997, a cloned sheep (“Dolly”) was produced when the nucleus of a mammary gland cell, a somatic cell, was transferred into an enucleated egg (Wilmot et al., 1997) by the process termed somatic cell nuclear transfer (SCNT).

The term cloning is misapplied to SCNT because the animals produced by the technology are genetic near copies and not genetic replicas of the animal donating the somatic cell. Genetic replicas require identical gene sequences (that is, DNA sequences) and identical patterns of gene expression. The pattern of gene expression and the gene sequence are both necessary for the genetic constitution or genotype of an individual and both are “genetic”. On the other hand, plant and bacterial clones are genetic replicas. Unfortunately, the erroneous use of “cloning” continues to be

perpetuated and remains a source of confusion and miscommunication. It has given rise to unwarranted expectations and over-inflated claims such as the possibility for duplicating a beloved pet, the performance of a champion thoroughbred horse, or the performance of elite production animals (see Conclusion 5). The NRC (2002) believed that it was pointless in trying to replace the term “cloning” with a more cumbersome phrase.

One other matter is the considerable confusion and obfuscation about reproductive cloning by means of SCNT and stem cell cloning. The ethical issues arising from reproductive cloning and stem cell cloning are quite different (National Research Council, 2002b).

The process of somatic cell nuclear transfer has the following steps:

1. Somatic cells from a desired donor are cultured in an artificial environment (in vitro). Mammary gland cells were used in the case of “Dolly”,
2. Oocytes (female germ cells) are obtained either after death or by surgery from the ovaries of ewes that have been treated hormone-impregnated vaginal tampons to cause multiple ovulations.
3. The nucleus of oocytes is removed by micro-suction.
4. DNA from the somatic cell is then transferred into the enucleated oocytes. Before doing this, the somatic cells are maintained for a period in nutrient poor medium to induce a state of metabolic quiescence. In addition, the cytoplasm of the enucleated oocyte retains factors that can reset, re-programme or de-differentiate the DNA in the somatic cell nucleus such that it can start to grow as an embryo.
5. Oocytes containing somatic nuclei are then fused in an electric current.
6. The resulting embryos are cultured for seven days either in the laboratory or in the ligated uterine tubes of a specially prepared female.
7. After this period of culture, the resulting blastocysts are transferred to the uterus of a surrogate dam where pregnancy may proceed to term.

Variations can be made to this protocol. These include "reverse-order" cloning, in which the order of enucleation and nucleus delivery is reversed and “zona free manual cloning methods” ("hand-made cloning") for embryonic and somatic cloning in cattle (Peura and Vajta, 2003).

#### *4.2.1 Problems with cloning*

The following excerpt is from the Internet site of the Human Genome Program of the U.S. Department of Energy Office of Science<sup>8</sup> and is dated July 2004:

“Reproductive cloning is expensive and highly inefficient. More than 90% of cloning attempts fail to produce viable offspring. More than 100 nuclear transfer procedures could be required to produce one viable clone. In addition to low success rates, cloned animals tend to have more compromised immune function and higher rates of infection, tumor growth, and other disorders. Japanese studies have shown that cloned mice live in poor health and die early. About a third of the cloned calves born alive have died young, and many of them were abnormally large. Many cloned animals have not lived long enough to generate good data about how clones age. Appearing healthy at a young age unfortunately is not a good indicator of long term survival. Clones have been known to die mysteriously. For example, Australia's

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<sup>8</sup> [www.ornl.gov/sci/techresources/Human\\_Genome/elsi/cloning.shtml](http://www.ornl.gov/sci/techresources/Human_Genome/elsi/cloning.shtml)

first cloned sheep appeared healthy and energetic on the day she died, and the results from her autopsy failed to determine a cause of death.”

The following statement summarises the findings of a committee on human reproductive cloning set up by the U.S. National Research Council (2002b). The statement was made by Dr I.L. Weissman, who chaired the committee, in an interview with Dr Norman Swan of ABC Radio on 29 November 2004:

“But what is important for the public to know, and I was the Head of the Panel that investigated it, is that attempts to do reproductive cloning of mice, sheep, pigs, cattle, all of these animals, as of 2001, of the 17,500 blastocyst, that is that ball of cells created by nuclear transfer, when they were implanted in the uterus, 99.2% of them died. Those that died late, that is, mid and late pregnancy, killed the mother about 50% of the time.”

The process of reproductive cloning is not fully controllable and the possibility of death in surrogate mothers is catastrophic. Accordingly, the American Medical Association and the American Association for the Advancement of Science have issued formal public statements advising against human reproductive cloning. Australia prohibits reproductive cloning in people.

As for cloning of livestock, the process is inefficient (Wells, 2005). About 6% of bovine embryos transferred to surrogate cows result in viable offspring. There are high losses during pregnancy, birth, shortly after birth and during calf-hood and adulthood. Losses during pregnancy result from placental failure. Placental insufficiency may have repercussions for the subsequent health of calves that survive.

Wells et al. (2004) report an annual mortality rate of at least 8% in cattle cloned from somatic cells between weaning and four years of age. A death rate of 8% is entirely unacceptable in farmed cattle. Reasons for death were variable. Deaths mainly resulted from euthanasia of animals with musculoskeletal abnormalities, which included severe contraction of flexor tendons and chronic lameness, particularly in milking cows. The picture was different for the progeny of clones where no deaths were recorded after weaning in a group of animals up to three years of age. Blood profiles and other indicators of overall physiological function such as growth rate, reproduction, rearing of offspring, and milk production were within expected ranges

In other studies, clinical diagnostic tests on cloned animals have given results within the normal range (Chavatte-Palmer et al., 2002; Matsuzaki and Shiga, 2002; Givoni et al., 2002). Furthermore, the reproductive performance of cloned heifers fell within expectations (Enright et al. 2002; Heyman et al., 2002). Clinical studies have appraised cloned calves as normal and healthy (Lanza et al., 2001).

All studies of the health and wellbeing of cloned livestock appear to have applied routine methods of clinical history, physical examination and laboratory testing. They have not been prefaced by a consideration of what the relevant questions or testable hypotheses about the health of cloned animals might be and how these could lead to relevant methods for appraisal. The threshold question is: Has genetic interference compromised the capacity of animals to function effectively in their proposed environment? This question links directly to the depiction of animal welfare as the state of an individual [animal] as regards its attempts to cope with its environment (Broom, 1986).

Two issues may be particularly relevant to the development of methods for appraising the health of cloned animals, and all B-D animals. First is that health extends beyond the absence of disease or infirmity. It includes physical, mental and social well-being (see definition earlier) and behavioural and physiological resilience in response to environmental, nutritional and social demands. Second is the importance of an animal's fit with its environment. Appraisals of the health of cloned animals undertaken within laboratories will not apply validly to every environment in which animal production occurs (see Conclusion 7).

#### 4.2.2 *Genetic hazards of cloning*

##### Imperfect reprogramming

In sexual reproduction, eggs and sperm fuse to form totipotential zygotes, which contain equal amounts of genetic material from both parents. Zygotes are referred to as totipotential because they are the ultimate source of all the cells in all the tissues of the body that arise through the process of differentiation. The DNA in zygotes is programmed for totipotentiality. By contrast, the DNA in body cells is programmed to express the particular characteristics of differentiated tissues such as muscle or nervous tissue. Cloning or somatic cell nuclear transfer produces live animals only when the DNA of the somatic cell has been adequately de-differentiated or re-programmed for the fused germ cell to become totipotential.

The process of re-programming is incompletely understood and involves aspects of epigenetic inheritance; see Box 3. Factors within the enucleated oocyte are involved (Hill, 2004). Incomplete or faulty re-programming is hypothesised to result from problems in epigenetic regulation (Trounson, 2001; Riek et al., 2003); see Box 3 for a description of epigenetic inheritance. Incomplete or faulty re-programming leads to uncoordinated and flawed development from the embryo onwards and a defective relationship with the uterine environment of the dam. Errors in re-programming can affect any gene; hence, the wide range of anatomical and functional disorders associated with nuclear cloning. Some errors of re-programming may occur only in particular tissues and only later in development (NRC, 2002b). The type of donor nucleus contributes to errors in re-programming and the abnormal pattern of gene expression seen in cloned animals (Jaenisch et al., 2005). According to Wells (2005), "future improvements in animal cloning will largely arise from a greater understanding of the mechanisms of reprogramming" (see Conclusion 10). Indeed, clarification around the basic biological questions thrown up by cloning may have more value to animal agriculture than cloning itself.

##### Defects in imprinting

The process called "imprinting" chemically marks the DNA from the dam and sire so that only one copy of a gene (either the maternal or paternal gene) is activated. The chemical mark on the DNA is usually methylation and imprinting is a form of epigenetic inheritance; see Box 3 for a description of epigenetic inheritance. The effect of imprinting is illustrated by the distinctive differences between hinnies, produced by breeding donkey jennets with horse stallions, and mules, produced by breeding horse mares with donkey jacks. For normal development, a developing embryo needs one set of chromosomes with a paternal imprint and one set with a maternal imprint. Imprinting errors in nuclear cloned animals may lead to faulty development and death and may be responsible for oversize in the foetus and placenta (Jaenisch et al., 2005). Imprinting is a form of epigenetic inheritance, see Box 3.

### **Box 3: Epigenetic inheritance**

Epigenetic inheritance refers to inheritance brought about by “alterations in DNA function without alterations in DNA sequence (Jones and Takai, 2001). According to the online encyclopaedia, Wikipedia ([http://en.wikipedia.org/wiki/Epigenetic\\_imprinting](http://en.wikipedia.org/wiki/Epigenetic_imprinting), accessed August 15, 2005), “epigenetic inheritance is the transmission of information from a cell or multicellular organism without that information being encoded in the nucleotide sequence of the gene. The study of epigenetic inheritance is known as epigenetics”.

“Epigenetic transmission of traits also occurs from one generation to the next in some organisms, although it is comparatively rare.”

So-called epigenetic inheritance systems may be involved in the evolutionary process. Epigenetic inheritance may also be involved in some human disease syndromes (Angelman syndrome and Prader-Willi syndrome).

Chromatin marking is one mechanism for epigenetic inheritance. Here proteins or chemical groups are attached to DNA and modify its activity. As for chemical groups, nucleotides in DNA (cytosine) can be methylated. Methylated DNA is copied during DNA replication.

#### Mitochondrial mixing

Mitochondria, the cell organelles involved in energy production, are derived from mothers as a result of “extrachromosomal inheritance”. In nuclear cloning, mitochondria can come from both the egg and the somatic cell. Mitochondria from the egg constitute the majority and problems are not seen (NRC, 2002a).

#### Differences in histocompatibility

Histocompatibility refers to the extent that tissue from one individual will be tolerated by the immune system of another individual. Histocompatible individuals will accept tissue grafts from one another. A lack of histocompatibility between transplanted nuclei (e.g. from one inbred strain of mouse) and the cytoplasm of oocytes (e.g. from a different inbred strain of mouse) can cause problems in growth and development (NRC, 2002b).

#### Somatic mutations

Somatic cells may contain DNA that has been damaged by environmental factors such as radiation. They may also harbour silent infections with viruses such as the herpesviruses. For this reason, somatic cells for nuclear transfer must be selected prudently and tested if possible for the presence of damaged or extraneous DNA.

#### Telomere shortening

Telomeres are DNA structures that stabilise the ends of chromosomes. They shorten with age and were thus regarded as a possible problem for nuclear cloning. This does not appear to be the case for sheep. Telomeres are rebuilt in cloned bovine embryos (NRC, 2002b).

#### Inactivation of the X-chromosome

Sex chromosomes determine the sex of animals. In mammals, females have two X chromosomes and males have an X chromosome and a Y chromosome, which is shorter. Females can reduce the production from genes in the X chromosome to the level observed in the Y chromosome. Cloned embryos of mice can do the same, indicating that cloned animals may not be troubled by problems associated with the activity of the X-chromosome (NRC, 2002b).

### **4.3 RNA interference**

The gist of this biotechnological intervention in animals is that small RNA molecules will interfere with gene expression and can, for example, switch genes off. RNA silencing may occur in nature where it suggested to act as a protective mechanism against viral infection (Downward, 2004).

Problems with the application of RNA interference technology to farm animals are highlighted by three papers that appeared over a short period in the Proceeding of the National Academy of Sciences of the United States of America. In the first, Golding et al. (2006) floated RNA interference as a means of protecting cattle against bovine spongiform encephalopathy (BSE). The thesis is that the prion gene, which is important in the genesis of BSE, can be switched off in cattle by RNA interference. The authors used lentiviral vectors for transgenesis. The other two papers (Steele et al, 2006; Zhang et al., 2006) show that the prion gene and its protein product are important in the development and differentiation of both nerve cells and blood cells. A generalisation made be made: RNA silencing can be listed as a hazard for the fitness of animals and this should be addressed as part of any research program into the development and application of this technology to animals.

## **5. A CHECKLIST OF GENETIC HAZARDS LINKED TO BREEDING TECHNOLOGIES**

As described earlier, the term hazard is associated with the potential of agents or situations to cause adverse effects or events. A hazard is a source of risk that does not necessarily produce risk. In this light, the interventions of biotechnology, genetic modification and cloning can be regarded as “initiating” or “inciting” hazards with a capacity for generating one or more consequent hazards within the genetic material of biotechnology-derived (B-D) animals. These are the “genetic hazards” and they may have an adverse effect on the normal structure and function of animals.

The usual primary concepts applying to disease can guide a consideration of when, where, how and why the physiological and behavioural stability and resilience of B-D animals may be undermined and lead to poor health and welfare. These primary concepts are (1) the cause or aetiology of disease or poor health, (2) the mechanisms involved with disease or poor health (the pathogenesis) and (3) the functional and structural consequences that are expressed as signs or symptoms of disease or poor health.

Unless other concurrent external causes are identified, the inciting cause (aetiology) of compromised physiological and behavioural stability, poor health and disease in B-D animals must be regarded as an unintended consequence of the biotechnological intervention and changes produced within the B-D animals. Established biological knowledge points confidently to mechanisms (pathogenesis) arising from induced defects and deficiencies in genes and their expression. These possible defects and deficiencies in genes and their expression constitute the genetic hazards for B-D animals.

The compilation of genetic hazards in Table 1 may be useful for establishing pathogenesis as a reference point for the surveillance and monitoring of health and welfare in B-D animals. Of necessity, this surveillance and monitoring must be based on observed functional and structural changes and on provoked physiological and behavioural responses (see Conclusion 9). Lifetime fitness of B-D animals is a

consideration and an innovative total diagnostic framework requires development (see Conclusion 9). Guidance here is available from methods used to assess the health and wellbeing of laboratory mice (Crawley, 1999; Lathe, 2004; van der Staay and Steckler, 2001).

**Table 1 – Genetic hazards associated with Biotechnology-derived animals.**

<b>Technology</b>	<b>Genetic hazard</b>	<b>Factors involved</b>
Selective breeding	Single gene disorders (inborn errors of metabolism)	Genetic drift, pre-existing faulty genes, population bottlenecks and the founder effect. Comprehensive list in Online Mendelian Inheritance in Animals at <a href="http://www.angis.org.au/Databases/BIRX/omia/">www.angis.org.au/Databases/BIRX/omia/</a> .
	Chromosome aberrations	
	Polygenic inheritance	Familial tendencies or and increased liability towards disease expressed as relatively high heritability
	Inbreeding depression	Decreased general fitness with insidious onset and difficult to diagnose. Results from a high frequency of mating between related animals.
	Compromised genotypic fit with environment	Best examples occur in milk producing and egg-producing animals when there is insufficient protein and energy in the feed supply.
Assisted breeding technologies	Artificial insemination and embryo transfer can facilitate the transmission of infectious disease. Comprehensive management of infectious disease operates for both artificial insemination and embryo transfer in cattle. There is some evidence that all manipulations making up assisted breeding technologies reduce viability of mouse embryos.	
Transgenesis	Insertional mutations	Random insertion of transgene may lead to unexpected interactions with pre-existing genes. Transgene may be introduced into the DNA sequence of pre-existing gene and destroy it.
	Transgene expression	The level, site and timing of expression of transgene is uncontrollable and differs each time transgenic animals are constructed. So-called epistatic gene effects may occur. The transgene product may be foreign to the animals and have unexpected effects. The transgene product may not be foreign to the animals but may be excessive and overtax mechanisms for maintaining physiological stability.
	Virus activity	Viruses used as vectors may change as a result of evolutionary forces.
	RNA interference	The gene that is switched off by small RNA molecules may be vital to fitness.

<b>Technology</b>	<b>Genetic hazard</b>	<b>Factors involved</b>
Cloning - Somatic cell nuclear transfer	Epigenetic effects	Epigenetic effects result from alterations in DNA function without alterations in DNA sequence. They can result from chemical changes to DNA (methylation) and may underlie imperfect reprogramming and defects in imprinting (see below).
	Imperfect re-programming	Failure of somatic cell to revert completely to totipotentiality. Errors in re-programming can affect any gene and thus lead to the wide range of anatomical and functional disorders associated with cloning.
	Defects in imprinting	Imprinting refers to chemical “marks” that distinguish between DNA from the dam and sire and lead to differences in gene expression . Errors in imprinting are common and cause problems.
	Mitochondrial mixing	Unlikely to be a risk.
	Histocompatibility differences	Problems arising from histocompatibility differences have been demonstrated experimentally in mice. [Histocompatibility refers to the degree that individual animals accept tissue grafts from one another.]
	Somatic mutations	DNA in somatic cells may be damaged, for example, by radiation or may contain viral DNA.
	Telomere shortening	Unlikely to be a risk.
	Inactivation of X chromosome	Unlikely to be a risk.

## 6. A PLACE FOR RISK ANALYSIS IN MANAGING THE WELFARE OF B-D ANIMALS?

A review of the Office of the Gene Technology Regulator (OGTR) and its covering legislation is in train. Information about this review (terms of reference etc.) can be found at <http://www.tga.gov.au/gene/gtreview.htm>. Risk analyses are routinely performed by the OGTR. NCCAW could provide advice and assistance on the nature of the risk analysis required for animal welfare under the umbrella of the Australian Animal Welfare Strategy – see Conclusion 13.

*Preliminary Note: The term risk analysis is used in different ways in different situations and, for this reason, is open to miscommunication. In the present paper it refers to models based on that originating from the Codex Alimentarius Commission of FAO/WHO. The idea of release assessment is included and comes from the risk analysis model for B-D animals of the Canadian Food Inspection Agency.*

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The process of risk analysis is the key to the management of food safety (FAO/WHO, 1997). It is also applied to other risks to human health and the environment (Presidential/Congressional Commission on Risk Assessment and Risk Management, USA (1997) and there are precedents for its general application to animal health and welfare. One precedent comes from reviews by the Scientific Steering Committee of the European Commission (2000, 2003).

Risk analysis has also been extended to the animal welfare aspects of biotechnology, genetic modification and cloning. Here it can provide the practical means for managing what has been termed “New Perception” debate on animal agriculture (Fraser, 2001) and its multitude of ethical, cultural, social, scientific and economic issues. Indeed, a paper by Van Reenen et al. (2001) framed a critical consideration of the adverse impacts of transgenesis around the concept of risk assessment. The possibility of applying risk analysis to the animal welfare aspects across the broad range of interventions in animals covered by biotechnology, genetic modification and cloning (B-D animals) has been realised in Canada. Moreau and Jordan (2005) describe the risk analysis framework used by the Canadian Food Inspection Agency to assess the risks to animal health that may operate in biotechnology-derived (B-D) animals. This framework includes the notion of “release assessment” which can aid in answering the question: When can B-D animals can move from the research and development phase and into general use?

As to the “New Perception” debate on animal agriculture, its elements have been described by Fraser (2001) and are shown in Box 4. The nub is the “urgent need for scientists and ethicists to avoid simply aligning themselves with advocacy positions and instead to provide knowledgeable research and analysis of the issues”. The Australian Animal Welfare Strategy has been instituted to address the issues highlighted by Fraser and to facilitate evidence-based policy. An indication of what constitutes evidence-based policy is shown in Box 5.

#### **Box 4: The “New Perception of animal agriculture” (Fraser, 2003)**

##### **From the abstract:**

A growing popular literature has created a “New Perception” of animal agriculture by depicting commercial animal production as (1) detrimental to animal welfare, (2) controlled by corporate interests, (3) motivated by profit rather than by traditional animal care values, (4) causing world hunger, (5) producing unhealthy food, and (6) harming the environment.

##### **From “implications”**

As it has unfolded to date, the New Perception debate has been disappointing intellectually, ethically, and politically: intellectually, because the debate has not resulted in a genuine understanding of how animal agriculture affects animals, the environment and the good of the public; ethically, because of the polemical nature of many of the accounts of animal agriculture has tended to polarize the debate and to prevent real ethical analysis of important issues; and politically, because this polarized debate has failed to create a climate of dialogue and consensus building. As a first step towards rectifying these problems, there is an urgent need for scientists and ethicists to avoid simply aligning themselves with advocacy positions and instead to provide knowledgeable research and analysis of the issues.

#### **Box 5: Evidence-based policy**

The following comes from the Urban Institute; a non-profit, non-partisan institute set up in the USA by President Johnson in 1968 to look at America’s cities and urban populations:

Evidence based policy is a rigorous approach that draws on careful data collection, experimentation, and both quantitative and qualitative analysis to answer three questions: What exactly is the problem? What are the possible ways to address the problem? And what are the probable impacts of each? A fourth question that figures into all public policy – What political and social values do the proposed options reflect? – is largely outside the scope of evidence-based policy. Nevertheless, hard evidence and analysis can bound the political battlefield, help build consensus, and identify the social and economic costs of different policy choices.

[http://www.urban.org/uploadedPDF/900636\\_EvidenceBasedPolicy.pdf](http://www.urban.org/uploadedPDF/900636_EvidenceBasedPolicy.pdf) - accessed 12 September 2005

Risk analysis is especially pertinent to Goal 2 of the Australian Animal Welfare Strategy, which is to “achieve sustainable improvements in animal welfare based on national and international benchmarks, scientific evaluation and research, taking into account changes in whole of community standards”. Goal 2 contains aims that are virtually the same as those found in risk analysis. The aims in Goal 2 are to:

- involve all stakeholders in ownership of the Australian Animal Welfare Strategy;
- maintain and improve the scientific basis for animal welfare standards;
- ensure that new knowledge gained through research on animal welfare is broadly communicated and adopted into national animal welfare standards; and to
- ensure formal processes for community involvement in the development and implementation of welfare standards.

When applied to food safety, risk analysis determines systematically what preventive actions can be taken to ensure that food does not lead to illness produced by food-borne organisms or toxins. It is an essential starting-point for quality management of food safety, including so-called Hazard Analysis Critical Control Point (HACCP) based systems.

When applied to animal welfare, risk analysis could determine systematically the sort of husbandry needed for animals in order to ensure their welfare and protect them from harms. In other words, the risk analysis process could underpin the development of animal welfare standards by providing an orderly approach to considering and

integrating scientific and other inputs. Risk analysis may provide appropriate processes for helping to reconcile objective facts and concepts about animals with the more subjective values people place on animals (see Conclusion 11).

In short, the application of risk analysis to B-D animals may have two related benefits (see Conclusion 11). These are to:

- protect the health and welfare of animals by specifying “concrete steps to monitor and prevent possibly adverse effects” (Van Reenen et al., 2001)
- maximise the benefits of biotechnology and to reward the research and development effort involved by minimising possible unintended and injurious consequences.

The Codex Alimentarius Commission (CAC) was created in 1963 by FAO and WHO to develop food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme. The principles that the CAC developed for risk assessment for food safety could be adapted for application to animal welfare (see Conclusion 12). These principles are set out as follows and are accompanied by comments to link them with animal health and welfare and with B-D animals.

1. Health and safety aspects of the Codex decisions and recommendations should be based on risk assessment as appropriate to the circumstance.  
*Comment: Animal welfare decisions should be based on risk assessment as appropriate to the circumstance.*
2. Food safety risk assessment should be soundly based on science, should include the four steps of the risk assessment, and should be documented in a transparent manner.  
*Comment: Animal welfare risk assessment should be soundly based on science, should include the four steps of the risk assessment, and should be documented in a transparent manner.*
3. There should be functional separation of risk assessment and risk management while recognised that some interactions are essential for a pragmatic approach.  
*Comment: Identical requirement for animal welfare.*
4. Risk assessments should use quantitative information to the greatest extent possible and risk characterisation should be presented in readily understood in a readily understandable and useful form.  
*Comment: The use of quantitative information may not be appropriate for animal welfare where much of the scientific knowledge is qualitative and conceptual rather than quantitative and where assessment is based on systematic observation.*

Three separate but integrated parts of risk analysis make up the “risk cycle”, a non-linear, fluid, dynamic and iterative process, which “requires both thought and action” (FAO/WHO, 1997). The three parts are risk assessment, risk management and risk communication and the labels used to differentiate them have very specific meanings as described by the Scientific Steering Committee (SSC) of the European Commission (2000) – see Figure 1.

**“Risk assessment** is a process of evaluation, including the identification of the attendant uncertainties, of the likelihood and severity of an adverse effect(s) / event(s) occurring to man or the environment [*substitute “animal” here*] following exposure under defined conditions to a risk source(s). A risk assessment comprises four steps: hazard identification, hazard characterisation, exposure assessment, and risk characterisation.

*Comment: The four steps of risk assessment may not be appropriate for all aspects of animal health and welfare. As for B-D animals, the methods of transgenesis and cloning explored to date have known shortcomings, including low efficiency, random integration of DNA into the genome and variable patterns of expression by transgenes (Niemann et al., 2005). As a result, a generic risk assessment will not be applicable in all instances. Every line of B-D animal may require separate assessment (see Conclusion 7 on diagnosis).*

**Risk management** is the process of weighing policy alternatives in the light of the result of a risk assessment(s) and of other relevant evaluations, and, if required, of selecting and implementing appropriate control options (including, where appropriate monitoring and surveillance activities).

*Comment: Risk management may necessitate adjustments to the nutritional, physical and social environments in which B-D animals are maintained. The match between animals and their environment is a time-honoured theme in animal husbandry. Breeds of animals exist because of their capacity to perform in particular environments.*

**Risk communication** is the interactive exchange of information and opinions throughout the risk analysis process concerning risk. It should involve not only risk assessors and risk managers, but also consumers and a wide range of other actual or potential stakeholders”. Risk communication is commonly misunderstood to consist of press releases or communiqués.

*Comment: Risk communication already occurs in animal-based agriculture. The commonplace information available on the behaviour of particular breeds in particular environments is a form of risk communication. The performance specifications developed for laboratory animals and hybrid strains of commercial poultry and are forms of risk communication. McCrea (2005) has explored risk communication in relation to B-D animals.*

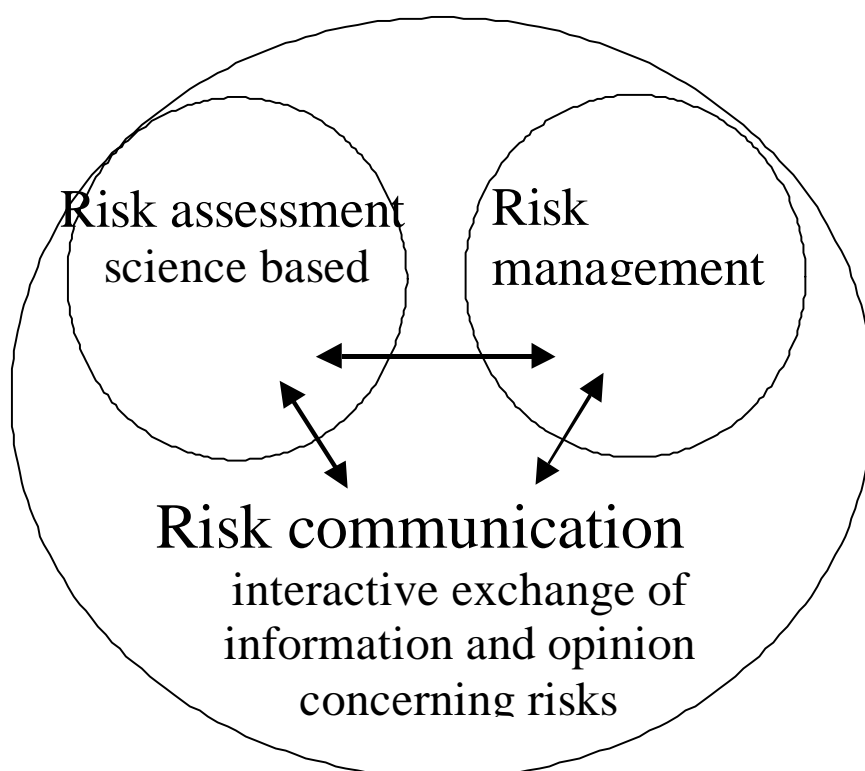
Risk assessment should be a scientifically based process. “A functional separation of risk assessment is necessary to ensure the scientific integrity of the risk assessment process and to reduce any conflict of interest between science policy considerations” (SSC, 2000). A clear distinction is made between hazards and risks. “Hazard” refers to inherent properties of an agent or situation that are associated with the potential of an agent or situation to cause an adverse effect(s)/event(s). “Risk” is a function of the probability and severity of an adverse effect/event occurring to man or the environment [*insert “animal” here*] following exposure to a hazard.

Risk assessments should take account of uncertainties and constraints in assessment. The formal processes available to deal with uncertainties were not applicable to the present risk assessment. On the other hand, benchmarks can be identified for the purpose of comparing risks and providing a sense of proportion or scale.

The Codex Alimentarius model of risk analysis emphasises the importance of input into risk management decisions of all interested parties who are likely to be affected by the decisions made. “These groups may include (but should not be limited to) consumer organizations, representatives of the food industry and trade, education and research institutions, and regulatory bodies”.

*Comment: The Australian Animal Welfare Strategy promotes the inclusion of all interested parties in the decision-making processes of risk management.*

**Figure 1 – Risk analysis framework redrawn from WHO**  
[www.who.int/foodsafety/micro/riskanalysis/en](http://www.who.int/foodsafety/micro/riskanalysis/en)



## 6.1 Release assessment

Release assessment is part of the risk analysis framework of the Canadian Food Inspection Agency for B-D animals (see Box 6). Release assessments look to the case for and against release of B-D animals into general use. They include “the risk to the B-D animals themselves and subsequent generations”.

### **Box 6: Release assessment (from Moreau and Jordan, 2005)**

A release assessment describes and quantifies the potential of a risk source (the animal or animal products) to release or introduce a hazard into an environment accessible to animal and human populations, and includes the risk to the B-D animals themselves and subsequent generations.

Release assessment involves consideration of the prevalence of the hazard, the point at which the hazard can be detected and the methods used to detect the hazard. The release assessment typically describes the type, amount, timing, and probability of the release of the hazard. In addition, the release assessment will include consideration of how these factors may change as a result of various actions, events or measures outlined in the release protocol. The various types of hazards – infectious, genotypic and phenotypic – dictate the variety of measures that need to be considered in the release assessment. In addition, all release assessments of B-D animals and their derived products must include consideration of the effects of animal waste products.

## **6.2 Risk equity**

Risk equity is a component of the risk analysis framework set out by Canadian Food Inspection Agency and may be relevant to the animal health and welfare aspects of B-D animals in Australia (see Conclusion 12). The Canadian Food Inspection Agency refers to “risk producer-beneficiaries”, “risk bearers” and “risk-benefit distribution”. Risk producer-beneficiaries are those that create the risk and benefit from it. Risk-bearers are those who bear the brunt of risk, either wittingly or unwittingly, and who would benefit from risk management. Operators of livestock enterprises using B-D animals would classify as risk bearers. Risk-benefit distribution describes the distribution and benefits of risk in society. The concept of risk equity may provide an important protection to those involved with animal production who should not be handicapped by B-D animals that either have health problems or are ill-fitted to their production environment (see Conclusion 12).

## 7. CONCLUSIONS

### *The Australian Animal Welfare Strategy*

1. The Australian Animal Welfare Strategy provides a nationally accepted context for making public policy decisions about the health and welfare aspects of B-D animals. The AAWS sets out an inclusive approach to ensure that the interests and concerns of all interested parties are considered. These parties include the public at large, the biotechnology industry and the agricultural industries which depend upon robust and adaptable animals maintained in good health and welfare.

### *An Australian review of the health and welfare aspects of biotechnology-derived (B-D) animals*

2. There are several pre-existing and formally accredited reviews of the animal health and welfare aspects of biotechnology-derived (B-D) animals. These come from the USA, the UK and Canada. Accordingly, Australia need not start de novo and undertake a similar public review. It may be necessary, however, to extract the key points from these other reviews and test whether (1) they are appropriate for Australia, (2) there are gaps in their coverage and (3) they have been referred to in policy processes already in train.

### *The possible role of B-D animals in Australian agriculture*

3. B-D animals have demonstrated their value in biomedical research; for example, in investigating prion diseases. They may also have application in producing particular proteins for the pharmaceutical industry. However, most current reviews are sceptical about the prospects for B-D animals in agriculture. Realistic prospects for the use of B-D animals in agriculture can be gauged if a reasoned and plausible case for feasibility in the face of known basic problems is presented and if a cogent process for accrediting the health of B-D animals can be followed.
4. An exercise in foresighting following the example set by the USDA Advisory Committee on Biotechnology and 21<sup>st</sup> Century Agriculture could be useful for exploring the possible role of B-D animals within the context of Australia.
5. The inaccurate but accepted application of the term cloning to somatic cell nuclear transfer (SCNT) should be borne in mind when assessing the possible role of B-D animals in Australian agriculture. Animals cloned by SCNT are genetic near copies not genetic replicas of the animal donating the somatic cell. The gene sequence may be the same but differences occur in gene expression. Both gene sequence and gene expression are “genetic”. Accordingly, claims about the possibility for duplicating a beloved pet, the performance of a champion thoroughbred horse or the performance of elite production animals can be regarded as dubious.

### *Evaluation of the health and fitness of B-D animals*

6. Accreditation of the health and welfare of B-D animals is required as re-assurance to the general public, to provide intelligible points of reference for the biotechnology industry and to protect Australian agriculture against a handicap of B-D animals that either have health problems or are ill-fitted to their production environment.
7. A fusion of the definition of health of the World Health Organization and the definition of animal welfare as the state of an individual as regards its attempts to cope with its environment can be used to frame assessments of the health and welfare of B-D animals. This fusion will facilitate the wealth of relevant knowledge found within comparative medicine, comparative pathology and integrative physiology. It also covers the need for an appropriate match between animals and their environments (the genotype-environment interaction). A key question is: Do appraisals of the health of B-D animals undertaken within laboratories and at research sites apply validly to every environment in which animal production may occur?
8. Genetic diversity within populations of animals is a significant biological contributor to their present and future health. Accordingly, an epidemiological or population focus may be called for when the health and welfare of B-D animals is being assessed.
9. A list of genetic hazards may be useful for establishing pathogenesis as a reference point for the surveillance and monitoring of health and welfare in B-D animals. Of necessity, this surveillance and monitoring must be based on observed functional and structural changes and on provoked behavioural and physiological responses. Lifetime fitness of B-D animals is a consideration and an innovative total diagnostic framework is required. Guidance here is available from methods used to assess the health and wellbeing of laboratory mice

### *Scientific research*

10. It is unfortunate that the prospect of imminent application to agriculture appears to have driven research and development into B-D animals in Australia. Research should continue for its value in clarifying biological questions that may contribute more to agriculture than B-D animals themselves. A multidisciplinary approach will be valuable. An innovation could be more emphasis on integrated or organismal physiology combined with behavioural science which will clarify interactions among body systems; for example, among the nervous, endocrine and immune systems.

### *Risk analysis*

11. The process of risk analysis presents itself as a practical method for managing the animal welfare aspects of B-D animals within the setting of the Australian Animal Welfare Strategy. Risk analysis can provide appropriate procedures for helping to reconcile objective facts and concepts about animals with the more subjective values people place on animals. It can specify the steps necessary to monitor and prevent adverse effects in B-D animals and thus allow the possible benefits of biotechnology.

12. In particular, the risk analysis procedures developed by the Codex Alimentarius Commission (CAC) could be adapted for application to animal health and welfare. CAC risk analysis includes the flexibility to be either specific or generic depending upon need and some guiding principles that could be re-worded for animal health and welfare. An addition may be use of the “release assessment” to help decide when B-D animals can move from the research and development phase and into general use. Release assessment for B-D animals has been described by the Canadian Food Inspection Agency. The issue of risk equity is likely to be important to allow for the success of the biotechnology industry and to protect Australian agriculture against the contingency that B-D animals may have health problems or are ill-fitted to their production environment.
13. A review of the Office of the Gene Technology Regulator (OGTR) and its covering legislation is in train. Information about this review (terms of reference etc.) can be found at <http://www.tga.gov.au/gene/gtreview.htm>. Risk analyses are routinely performed by the OGTR. NCCAW could provide advice and assistance on the nature of the risk analysis required for animal welfare under the umbrella of the Australian Animal Welfare Strategy (AAWS). The AAWS seeks to ensure formal processes for community involvement in the development and implementation of welfare standards.

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