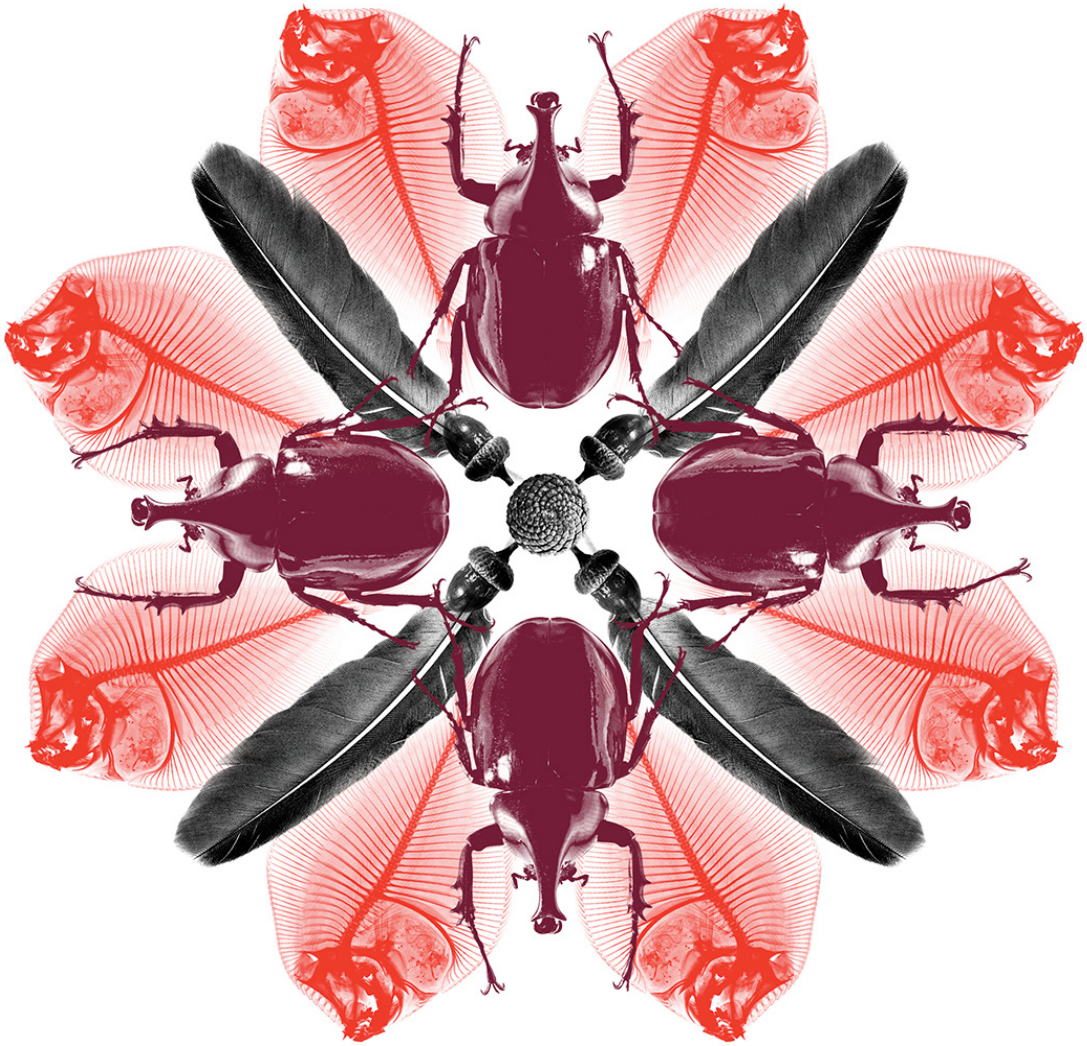
Gamma irradiation as a treatment to address pathogens of animal biosecurity concern

Final policy review

November 2014



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Contents

Glossary

ALOP	appropriate level of protection
BSA	bovine serum albumin
BVDV	bovine viral diarrhoea virus
Ci	Curie
CJD	Creutzfeldt-Jakob disease
Code, the	Australia New Zealand Food Standards Code
Codex	Codex Alimentarius Commission
CsCl	cesium chloride
department, the	Australian Government Department of Agriculture
Dmax	maximum dose received by any part of the treated material
Dmin	minimum dose received by any part of the treated material
DNA	deoxyribonucleic acid
DS	sterilisation dose
dsDNA	double-stranded DNA
dsRNA	double-stranded RNA
FAO	Food and Agriculture Organization of the United Nations
FBS	foetal bovine serum
FCS	foetal calf serum
FMD	foot-and-mouth disease
FMDV	foot-and-mouth disease virus
Gy	gray
HIV	human immunodeficiency virus
HSV	herpes simplex virus
IAEA	International Atomic Energy Agency
IBR	infectious bovine rhinotracheitis

kGy	kilogray
krad	Kilorad
MED	minimum effective dose
MEM	minimal essential medium
Mrad	Megarad
N	bio-burden
NssRNA	negative sense single-stranded RNA
OIE	World Organisation for Animal Health
PBS	phosphate-buffered saline
PFU	plaque-forming unit
PPV	porcine parvovirus
PrP	prion protein
R	Roentgen
rep	roentgen equivalent physicals
RNA	ribonucleic acid
S	radiation sensitivity
SAL	sterility assurance level
SI	International System of Units
SPF	specific pathogen free
SPS Agreement	Sanitary and Phytosanitary Agreement
ssDNA	single-stranded DNA
ssRNA	single-stranded RNA
Sv	Sievert
TCID	tissue culture infective dose
TSE	transmissible spongiform encephalopathy
UV	Ultraviolet
WHO	World Health Organization

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Summary

Gamma irradiation is currently accepted by the Department of Agriculture (the department) as a biosecurity treatment for a range of products of animal origin including animal fibres, aquatic animal feed, artefacts, hides, laboratory reagents and specimens, pet food, skins and veterinary therapeutics. This is especially the case where a product does not meet Australia's biosecurity requirements, or the product has not been adequately processed or tested to address Australia's biosecurity concerns, or where the manufacturer is unable to provide the department with sufficient information to complete the risk assessment. Irradiation is not intended to be a replacement to a production process, treatment or import policy but as an adjunct to address biosecurity concerns.

The department accepted gamma irradiation at 25 kilogray (kGy) as a biosecurity treatment in the early 1980s. In the early 1990s, the dose was increased to 50 kGy, based on the available scientific literature and expert opinion at the time.

This review of gamma irradiation as a treatment to address pathogens of animal biosecurity concern has been undertaken to identify and recommend appropriate irradiation doses needed to inactivate bacterial, fungal and viral pathogens, and parasites (cestodes, nematodes, protozoa and trematodes) in products of animal origin. Although the focus of the review is to address pathogens of animal biosecurity concern to Australia, it also considers the effectiveness of gamma irradiation against animal pathogens in general. This information will be used in conjunction with other available information such as the country, and species, of origin of the product, and the processing of each ingredient and/or the final product, to enable the department to determine more accurately the required irradiation dose when used as an animal biosecurity treatment. While 50 kGy remains the current standard, this review may also facilitate the use of lower levels of irradiation that still protect Australia's favourable animal health status in cases where the pathogens of concern are known. There may also be instances where a dose higher than 50 kGy may be required for products assessed as likely to be contaminated with significant amounts of pathogens of animal biosecurity concern.

All food, imported or domestic, intended for human consumption, must comply with the Australia New Zealand Food Standards Code (the Code). The option of irradiation is not available as a biosecurity import measure for food for human consumption unless supported by the Code. This review did not specifically deal with irradiation treatment to address human health or food safety concerns other than where data on the effectiveness of irradiation on human pathogens can be extrapolated to pathogens of animal biosecurity concern or where irradiation of a product may have a negative effect on animal health (for example, pet food). This review also does not specifically deal with pests and pathogens of plants.

The review makes the following recommendations on gamma irradiation to address animal biosecurity concerns:

The sterility assurance level

- The dose should be based on the likely bio-burden of each viable pathogen in the product.
- The sterility assurance level (SAL) should be set at 10^{-6} for each pathogen of concern unless an alternative SAL is established, through a risk analysis, for the specific pathogen and product.
- It is generally assumed that tissues used in the manufacture of biological products such as vaccines would be derived from healthy sources and that in most circumstances, the bio-burden is close to zero.
- An instance where the bio-burden is anticipated to be higher than zero would be in a situation where infected tissue may be imported into a laboratory for further research. In these situations, the bio-burden in the infected tissue must be considered on a case-by-case basis and a higher dose may be recommended. For example, if the the bio-burden is 10^3 plaque-forming units per gram (PFU/g), a nine log reduction in titre is required to achieve the SAL. If the D_{10} for the pathogen is 5 kGy, a dose of 45 kGy is required[1].

Validation

- Validation of the irradiation process, including assurances that treatment is applied both properly and consistently, is necessary especially for non-homogenous products, radiodense materials or high biosecurity risk products (based on country and species of origin and end use).

Product safety

- In accordance with S.48AA(4) of the *Quarantine Act 1908*, the department advises applicants to seek advice from service providers about the possible impact of irradiation on their product and only issues an import permit once the applicant agrees to irradiation as the alternative treatment.
- Recently, chronic leucoencephalomyelopathy in cats in Australia was most likely linked to the irradiation of either the pet food or its packaging. Subsequently, the department does not provide irradiation as a treatment option for cat food.
- For other products and except in exceptional circumstances such as specific pathogen free settings, irradiation as a biosecurity option should not be supported if those products are likely to be consumed as a significant portion of an animal's diet (for example, kibble).

[1] The D_{10} or D value is defined on page 10

General principles of irradiation treatment as a biosecurity measure

- Irradiation treatment is not intended to be a replacement to a production process, treatment or import policy but as an adjunct to address animal biosecurity concerns.
- Approval to use irradiation as a animal biosecurity option to address exotic disease concerns should take into consideration product parameters relevant to the effectiveness of irradiation (for example, radiodensity of materials, homogenecity of product), environmental factors (for example, water and oxygen levels), and if necessary, the animal health status of the country of origin, other processing treatments and certification confidence.
- Where irradiation treatment is considered necessary to address animal biosecurity concerns, an irradiation dose of 50 kGy should continue to be used unless otherwise determined by the biosecurity risk assessment of the import application.
- A risk assessment of the import application may determine that there are only a few animal pathogens of concern. In this case, the assessment may determine an irradiation dose lower than 50 kGy based on D_{10} values and the SAL.
- The risk assessment may occasionally determine that, based on D_{10} values and the SAL, a dose higher than 50 kGy is required for products assessed as likely to be significantly contaminated with pathogens of animal biosecurity concern.

Viruses

- Subject to the general principles above, the maximum D_{10} value as listed for the relevant virus species (see Table 8, Appendix 3) may be used in association with the recommended SAL to determine an appropriate gamma irradiation dose.
    - In the absence of reliable data for the specific virus species, the maximum D_{10} value for the family may be used as described in Table 2.
    - Where there is more than one viral pathogen of animal biosecurity concern, the maximum D_{10} value of the most radio-resistant virus should be used.

Bacteria and fungi

- Subject to the general principles above, radiation doses required to manage animal biosecurity issues associated with most viruses of concern in products will also address those associated with bacteria and fungi.
    - Where it is only necessary to address animal biosecurity issues associated with a specific bacterial species, the maximum value (see Table 9, Appendix 3) for that species could be used in association with the recommended SAL to determine an appropriate dose.

- If the specific bacterial species is not listed, the maximum D_{10} for the bacterial genus should be used.
- Should a fungal species be identified as a animal biosecurity concern, and in the absence of specific data for the organism or its genus (see Table 4), a D_{10} value of 2.90 kGy could be used in association with the recommended SAL to determine an appropriate dose.

TSE agents

- Irradiation is not considered an effective treatment and is not recommended.

Foodborne parasites

- Subject to the general principles above, radiation doses required to manage animal biosecurity issues associated with viruses in products will also address animal biosecurity issues associated with parasites.
  - Should a parasite be identified as a biosecurity concern, a dose of 25 kGy is recommended.

1 Introduction

Australia's biosecurity policy

Australia's biosecurity policies aim to protect Australia from risks that may arise from exotic diseases and pests entering, establishing or spreading, thereby threatening Australia's unique flora and fauna, as well as those agricultural industries that are relatively free from serious diseases and pests.

The Australian Government Department of Agriculture (the department) is responsible for developing and reviewing biosecurity policy for the importation of animals and their products. This is done through a science-based risk evaluation process. At the completion of the process and following consideration of stakeholder comments, the department is then responsible for implementing the import protocol, including any risk management measures.

Australia's science-based risk analysis process is consistent with Australian Government policy and Australia's rights and obligations under the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (also known as the SPS Agreement).

Australia implements a risk-based approach to biosecurity management. This approach is expressed in terms of Australia's appropriate level of protection (ALOP), which reflects community expectations through government policy and is currently aimed at reducing these risks to a very low level, but not to zero.

If the risks exceed Australia's ALOP, risk management measures are proposed to reduce the risks to an appropriate level. However, if it is not possible to reduce the risks to an appropriate level, then no trade will be allowed.

Background

This review of gamma irradiation as a biosecurity treatment to address pathogens and parasites of animal biosecurity concern has been undertaken to identify and recommend appropriate irradiation doses needed to inactivate bacterial, fungal and viral pathogens, and parasites (cestodes, nematodes, protozoa and trematodes) in products of animal origin. Previously, the department accepted a standard level of 50 kilogray (kGy) to address most pathogens and parasites of concern. This review will enable the department to determine more accurately the required irradiation dose. While 50 kGy remains the current standard, this review may also facilitate the use of lower levels of irradiation that still protect Australia's favourable animal health status in cases where the pathogens of concern are known. There may also be instances where a dose higher than 50 kGy may be required for products assessed as likely to be contaminated with significant amounts of pathogens of animal biosecurity concern and/or pathogens with very high D_{10} values.

Scope

The review considers the following:

- how sterilisation by gamma irradiation works
- the effectiveness of gamma irradiation against microorganisms as an animal biosecurity treatment option, and
- appropriate gamma irradiation doses needed to inactivate bacterial, fungal and viral pathogens, and parasites of animal biosecurity concern in products.

The review does not specifically deal with irradiation treatment to address human health or food safety concerns other than where data on the effectiveness of irradiation on human pathogens can be extrapolated to pathogens of animal biosecurity concern or where irradiation of a product may have a negative effect on animal health (for example, pet food). This review also does not specifically deal with pests and pathogens of plants.

Gamma irradiation and biosecurity

Gamma irradiation has been used worldwide for many years to address a range of biosecurity, food and pharmaceutical safety issues. In general terms, the more complex the organism (i.e. the larger the chromosome structure), the greater the effect of gamma irradiation.

Very low doses (0.2–0.7 kGy) are used to sterilise insects such as the fruit fly; moderate doses (~10 kGy) are used for some foods to decrease the level of vegetative bacteria (that is, bacteria that are in the growth and reproductive stage); higher doses are used where greater levels of assurances on freedom from contamination are needed. In particular, 25 kGy or higher is routinely used to sterilise medical equipment and pharmaceuticals (also known as health care products) where 'bacterial sterility' is required. Viruses are considerably more resistant to irradiation than are vegetative bacteria. Animal viruses that are exotic to Australia are of particular concern from a biosecurity perspective and thus require higher irradiation doses.

The minimum sterilisation dose (DS) for health care products to achieve the desired sterility assurance level (SAL) is usually determined through the use of bacterial bio-burden based validation methods. The desired SAL is usually set at 10^6, which provides an assurance that there is less than one chance in a million of viable contamination in any one unit. A minimum DS of 25 kGy is typically used, although there are numerous products identified by the healthcare industry that require a DS of up to 40 kGy.

Australia's biosecurity policy and the use of irradiation

The purpose of the *Quarantine Act 1908* is to prevent the introduction, establishment or spread of pests and diseases of biosecurity concern. Australia is free of most serious animal diseases affecting other countries. The *Quarantine Proclamation 1998*

refers to the level of biosecurity risk and the imposition of appropriate conditions that the Director of Animal and Plant Quarantine must consider prior to granting an import permit.

Gamma irradiation is routinely approved by the department as a biosecurity treatment for a range of products of animal origin, including aquatic animal feed, animal fibres, artefacts, laboratory reagents and specimens, pet food, skins and hides, and veterinary therapeutics. This is especially the case where a product has not been adequately processed to address Australia's animal biosecurity concerns, or where the manufacturer is unable to provide the department with sufficient information to complete the risk assessment.

Irradiation is not intended to be a replacement to a production process, treatment or import policy but as an adjunct to address biosecurity concerns.

Gamma irradiation, at a dose of 25 kGy, has been in use since at least 1985 by the department and probably much earlier. In the 1980s and early 1990s, based on the available scientific research at the time and expert advice, including from the Plum Island Animal Disease Center in the United States and the Australian Animal Health Laboratory, an irradiation dose of 50 kGy was progressively implemented by the department to address viruses of animal biosecurity concern in products on arrival in Australia. By the mid-1990s, 50 kGy had become the standard to address all animal biosecurity concerns.

At that time, the commercial irradiation facilities in Australia were set up to irradiate product, on a batch basis, at 25 kGy. To achieve the higher dose of 50 kGy, the product had to be irradiated twice. Modern irradiation facilities are now more flexible with their ability to deliver a specific dose, and it is acknowledged that 50 kGy may be excessive for some products and pathogens, and that there may also be pathogens that require doses in excess of 50 kGy to achieve an appropriate SAL.

Heat treatment, gamma irradiation and food safety

Detailed consideration of the food safety aspects of irradiated food is outside the scope of this review and the *Quarantine Act 1908*. However, for completeness, a brief review is presented here.

Heat treatment and irradiation are options for managing the potential biosecurity risks of pests and diseases. Both treatments have a potential effect on the characteristics of some foods. With all materials, free radicals are produced that combine to result in a change in the product. The interaction of irradiation with food produces changes that are usually limited to odour, taste and colour although there may also be an effect on nutrient and vitamin levels and the production of radiolytic products.

Irradiation has been used in the food industry in many countries in a range of products to extend the shelf life and improve the keeping properties of food by reducing the population of bacteria in the finished product (see the Australia New Zealand Food Standards Code, Standard 1.5.3, for more information: http://www.comlaw.gov.au/Details/F2009C00895, accessed 24 November 2011).

The food industry has made little practical use of irradiation processing largely due to consumer concerns. Spices and seasonings remain the main products irradiated on a significant scale, although the quantity and range of products approved for irradiation, including ready-to-eat meat and poultry products and fruit and vegetable products, are gradually increasing.

It is acknowledged that irradiation can have dose-related effects on certain vitamins and other nutrients and it produces peroxides and other radiolytic by-products, some of which may be toxic and/or carcinogenic. However, the same is true for thermal processing and possibly other food production technologies. There is no convincing evidence that irradiation of food is any more detrimental than thermal processing, although irradiation of pet food for cats may present a unique case.

The World Health Organization (WHO) reviewed an extensive number of animal feeding studies, primarily on dogs and laboratory rodents, conducted between the late 1950s and early 1980s (WHO 1994). It found that many of the 400 or more studies reviewed had significant design and other deficiencies, but it was able to conclude that the safety studies, including trials in human volunteers, did not demonstrate any deleterious effects linked to the consumption of irradiated food. Also, irradiated foods are generally nutritionally equivalent to non-irradiated foods that are subjected to normal processing.

The wholesomeness of irradiated food has undergone significant research, testing and evaluation over more than 50 years. For example, the 1980 Joint Food and Agriculture Organization (FAO)/International Atomic Energy Agency (IAEA)/WHO Expert Committee on the Wholesomeness of Irradiated Food concluded that the 'irradiation of any food commodity up to an overall dose of 10 kGy presents no toxicological problems' (WHO 1981). The Codex Alimentarius Commission (Codex) adopted this principle in 1983 as the Codex General Standard for Irradiated Foods (see www.codexalimentarius.net/download/standards/16/CXS_106e.pdf, accessed 16 February 2011).

Extensive research undertaken by the United States Army in the 1960s also supported the safety of irradiated food. After several irradiated animal feed studies (McDowell and Raica, Jr. 1962), the United States Army Surgeon General concluded in 1965 that foods irradiated with up to 56 kGy with a cobalt-60 source of gamma irradiation were safe and nutritionally adequate (Steele 2000; United States. Congress. Joint Committee on Atomic Energy. Subcommittee on Research, Development, and Radiation 1965). WHO (1994) supported this conclusion.

The wholesomeness of food irradiated with high doses (i.e. above 10 kGy) has also been supported by the report of a joint FAO/IAEA/WHO Study Group into high dose irradiation (WHO 1999) that concluded 'food irradiated to any dose appropriate to achieve the intended technological objective is both safe to consume and nutritionally adequate'. WHO (1999) concluded that 'no upper dose limit need be imposed' and stated that irradiated foods were deemed wholesome throughout the dose range from below 10 kGy to above 10 kGy.

2 Technical information

Radiation sources

The three ionising radiation sources typically used commercially for sterilisation are gamma, x-ray and electron beam, but as only gamma irradiation has been used in Australia for 30 years as a biosecurity treatment option, gamma irradiation is the focus of this review. However, for completeness, a comparison with x-ray and electron beam irradiation is also presented.This chapter also aims to provide an understanding of ionising radiation in general including its interaction with microorganisms and products.

Radiation may be grouped into two categories—electromagnetic and particle radiation.

Electromagnetic radiation

Electromagnetic radiation sources include gamma, microwave, ultra wave and X-ray. Gamma and X-rays are similar in nature, differing only in frequency and origin.

The radioisotopes typically used for gamma sterilisation processes are cobalt-60 (60Co) and caesium 137 (137Cs). Cobalt-60 is used more commonly in industrial processing due to its better penetrating power and availability. It decays to become non-radioactive nickel with the emission of two high energy gamma rays (1.17 and 1.33 MeV) and a low energy electron particle (0.318 MeV). Gamma rays have considerable penetrating ability because they have no mass and carry no charge (Hansen and Shaffer 2001; Lambert 2004).

Gamma irradiation is a flexible, simple, efficient and effective sterilisation method for large volumes of high or low density product including that product in its final packaging. Up to 95% of gamma raditation is available for use. The dose delivered within the target product is relatively uniform although treatment can be slow. However, radioactive sources such as Cobalt-60 used for gamma irradiation are dangerous requiring heavy shielding. The emission of an X-ray from an atom is produced by bombarding a heavy metal target with fast electrons in an accelerator. X-ray photons are also electromagnetic radiation sources with no mass and no charge and have considerable penetrating ability. X-ray ionising radiation requires much less shielding and the X-ray tube can also be turned off when it is not in use. Although X-rays can also be an effective sterilization method for large volumes of high density product, its use has been limited because the conversion of electrons to X-rays is very inefficient requiring high electricity consumption which can be expensive.

Particle radiation

Although there are many particles of importance in radiation biology (for example, α, β, meson, neutrino, neutron and positron), the β particle (or electron) is the only particle currently applicable to sterilisation. Electrons can arise from the decay of an isotope and have a low mass and single negative charge. The electron particles

comprising the electron beam are produced using an electric-powered machine source and have low penetration properties. The low penetration means that the width and density of products that can be irradiated are limited. Electron beam is therefore effective for the sterilisation of low density or small, uniformly packaged product but has limited application for other types of product. Advantages are that electric beams can be turned on only as needed, do not require replenishment of the source and there is no radioactive waste. However, they require high electric power consumption, are complex and potentially high maintenance.

Ionising radiation units

Measurement in radiation treatment is based on the amount of energy deposited in the material being treated and is referred to as the absorbed dose. Ionising radiation doses may be quoted as gray (Gy), kilogray (kGy), rad, kilorad (krad) or megarad (Mrad).

A rad is the original unit developed for expressing absorbed dose, which is the amount of energy from any type of ionising radiation (for example, α, β, gamma, neutron) deposited in any medium (for example, air, tissue, water). A dose of one rad is equivalent to the absorption of 100 ergs (a small but measurable amount of energy) per gram of absorbing material. The rad has been replaced by the gray in the International System of Units (SI); 1 gray = 100 rad. A gray is expressed in terms of absorbed energy per unit mass; 1 gray = 1 joule/kilogram = 100 rad. One megarad equals 10 kGy. Further information is presented in Appendix 1 (Terminology) and Appendix 2 (Conversion formula).

Action of irradiation

The absorption of high energy radiation results in ionisation within the treated material. Ionisation is the transformation of uncharged or stable individual atoms or molecules to a charged or unstable state. With particle radiation (for example, electron-beam), ionisation within the treated material is due to the direct interaction of the charged particles with the matter, dislodging both ions and individual atomic particles. With electromagnetic radiation (for example, gamma and X-ray), ionisation in the material is due to the indirect interaction of the photons with atoms causing electrons to be ejected (see Figure 1).

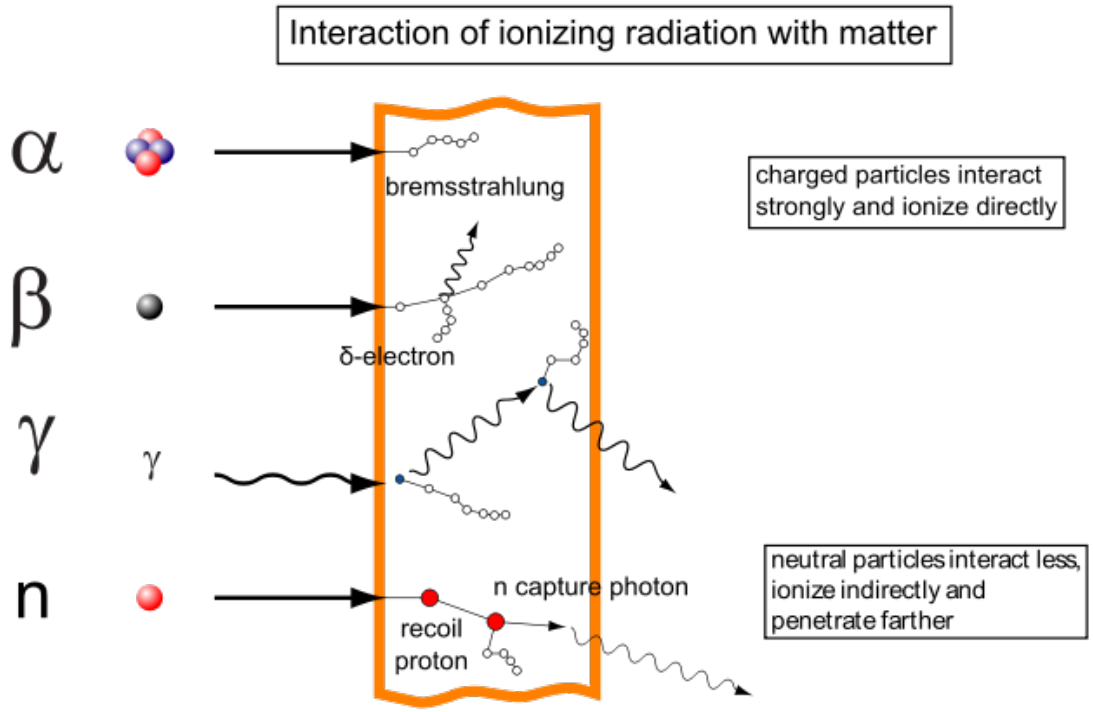

Figure 1. Interaction of ionising radiation with matter

(Source: Hanson and Shaffer 2001)

All parts of the treated material are not subjected to equal amounts of the ionising energy as passage through the material can be via discrete 'tracks' with localised areas along the tracks subjected to intense energy and other areas only slight alteration. Along these tracks, photons of energy ionise the material and also produce free radicals (i.e. atoms with an unpaired electron) and excited atoms. The breaking of chemical bonds and the formation of free radicals produces changes to both the treated material and microorganisms (Hansen and Shaffer 2001). A detailed explanation of the action of ionising radiation including target theory can be found in the online educational article about ionising radiation by Grossweiner (2009), available at www.photobiology.com/educational/len/part2.htm (accessed September 2011).

The action of irradiation on microorganisms is due to a variety of physical and biochemical effects although the major factor in loss of viability appears to be its effect on the deoxyribonucleic acid (DNA) molecule. Sensitivity to irradiation is determined by the chromosome volume of an organism. For example, organisms with higher chromosome volume such as plants and animals are more sensitive to irradiation compared to microorganisms. Differences in irradiation sensitivity may be due to an ability to repair nucleic acid damage rather than an inherent irradiation resistance of the nucleic acid (Hansen and Shaffer 2001; Lambert 2004).

Measurement of inactivation

The D_{10} value, sometimes referred to as the D value, is a measure of the treatment required to inactivate 90 per cent (i.e. a one log reduction) of the organisms present or to reduce the microbial population to one-tenth its number. For irradiation, the value is measured as the irradiation dose required to achieve a one log reduction in the titre (i.e. population) of the organism.

The use of D_{10} for irradiation treatments should not be confused with its use for thermal processes such as cooking, where the D_{10} is measured as the time required at a specific temperature to achieve a one log reduction.

The effect of ionising radiation on a biological system is quantified by the radiation sensitivity (S), which is defined as the reciprocal of the radiation dose required to cause a certain fractional change in a selected property. Some inactivation studies using irradiation express their results as D_{37} values, which indicates the irradiation required to reduce viable organisms to approximately 37 per cent (i.e. inactivate 63 per cent of the microbial population). S is often measured as $1/D_{37}$.

To facilitate comparison of inactivation studies, this review will convert and express D_{37} values as D_{10} values and all radiation doses in kGy. The exact reduction of microbial levels for D_{37} values is 36.79 per cent. The formula to convert quoted D_{37} values into D_{10} values is:

$$D_{10} \text{ value} = D_{37} / (-\log (0.3679)) \qquad [1]$$

Refer to Appendices 1 and 2 for further information.

Effect on products and product safety

The 1980 Joint Expert Committee on the Wholesomeness of Irradiated Food concluded that ‘irradiation of any food commodity up to an overall dose of 10 kGy presents no toxicological problems’ (WHO 1981).

This was adopted in 1983 as the *Codex General Standard for Irradiated Foods*. The applications of irradiation for food requiring doses less than 10 kGy include the elimination of vegetative bacterial pathogens from foods such as meat, poultry, fish and fresh fruits and vegetables; the inhibition of sprouting in potatoes and other tubers; the disinsection of grains and dried fruits such as dates and figs; extension of the shelf-life of refrigerated foods; and the treatment of certain foods. The beneficial results of food irradiation include the improvement of the hygienic quality of certain foods and the reduction of post-harvest losses.

Much higher irradiation doses may be used for sterilisation of food (for example, for immuno-compromised hospital patients). For example, the Netherlands permits a dose of 75 kGy for this purpose (WHO 1999). After several irradiated animal feed studies, the United States Army Surgeon General concluded in 1965 that foods irradiated with up to 56 kGy with a cobalt-60 source of gamma irradiation have been found to be wholesome, i.e. safe and nutritionally adequate (Steele 2000; United

States. Congress. Joint Committee on Atomic Energy. Subcommittee on Research, Development, and Radiation 1965).

In comparison to thermally sterilised foods, the extent of chemical change in radiation-sterilised foods is relatively small and uniform. Microorganisms are destroyed primarily because hydroxyl radicals formed within their cells react with the base and sugar moieties of DNA, which in part results in breakage of sugar-phosphate bonds and loss of the replication function. Micronutrients will be degraded to an extent that will depend on both their ability to compete against other major constituents for the primary radicals, and upon the irradiation conditions, including dose. Sensory attributes, such as colour, flavour and texture, will similarly be affected. Package functionality might be favourably or unfavourably affected by the competition between bond-breaking and bond-making (WHO 1999).

The macronutrients—carbohydrates, fats and proteins—are not significantly altered in terms of nutrient value and digestibility by irradiation treatment. Some of the micronutrients—vitamins—are susceptible to irradiation, but the extent depends on the composition of the food and on processing and storage conditions. Thiamine is the most sensitive to irradiation. For sensory qualities to be retained, food products (except dry products) irradiated to doses above 10 kGy will require irradiation in the absence of oxygen and at cryogenic temperatures. However for high dose irradiation to decontaminate dry commodities (with doses up to 30 kGy), low numbers of irradiation-resistant microbial cells may survive (WHO 1999).

The Codex General Standard states that the only radiation sources that are suitable for irradiation processing of food are high energy photons from radioisotope sources (cobalt-60 or caesium-137) and machine sources with accelerated electrons with energies up to 10 MeV or X-rays with energies up to 5 MeV (Farkas 2006).

Accelerated electrons have low penetrability with a practical penetration of only 3.9 cm for 10 MeV electrons in high moisture food. Gamma rays and X-rays have high penetrability facilitating treatment of product even in pallet-size containers. According to Farkas (2007), except for different penetration, the effects of electromagnetic ionising radiations and electrons are equivalent in food irradiation.

The radioisotope cobalt-60 is produced from metallic cobalt-59 which, when inserted into specifically designed nuclear power reactors, absorbs neutrons. The activated metal does not need any waste refinement treatment, and is doubly encapsulated as rods or discs in stainless steel casings before being released to radiation facilities.

The gamma rays emitted from cobalt-60 are between 1.3 and 1.33 MeV. These are well below the thresholds for photonuclear activation of any chemical element. Thus, no radioactivity can be induced in the exposed food.

The joint FAO/IAEA/WHO Study Group into high-dose irradiation concluded that food irradiated to any dose appropriate to achieve the intended technological objective is both safe to consume and nutritionally adequate. No upper limit was imposed, but the Study Group stated that irradiated foods are deemed wholesome throughout the dose range from below 10 kGy to doses above 10 kGy (WHO 1999).

Studies have confirmed the general safety of irradiated food at doses typically used for product for human consumption (i.e. <10 kGy). Proteins in particular are not significantly altered in terms of nutrient value and digestibility by irradiation treatment. However, there is evidence of significant reductions in some vitamins (particularly thiamine and vitamins A and E) and other nutritional factors, and an increase in lipid oxidation in animal feeds at doses >25 kGy. The extent of these effects depends on the composition of the food and processing and storage conditions, and the effects may affect the organoleptic qualities of the feed. Because of this and the evidence that irradiated pet food is potentially harmful to cats, irradiation as a biosecurity option should not be supported if those products are likely to be consumed as a significant portion of an animal's diet (for example, kibble).

Gamma irradiation and pet food safety

One of the many products that represent a potential biosecurity risk to Australia is pet food. Imported pet food, although eaten primarily by dogs and cats, may be inadvertently consumed by backyard poultry or wild birds, domesticated or feral pigs, and other domesticated or wild animals. If contaminated, it could introduce exotic animal diseases into Australia.

Pet foods usually contain a range of animal ingredients. Before approving import, the department assesses each application to ensure that the final product is not likely to be contaminated with pathogens or parasites of biosecurity concern. This assessment is based on the country and species of origin of each ingredient and the processing of each ingredient and/or the final product.

A pet food ingredient (or the final product) is considered acceptable if it is heat treated sufficiently to address Australia's animal biosecurity concerns. For products that do not meet requirements or where the manufacturer is unable to provide the department with sufficient information to complete the assessment, importers may be given the option to irradiate some pet foods to address outstanding biosecurity concerns.

In accordance with S.48AA (4) of the *Quarantine Act 1908*, the department advises applicants to seek advice from service providers about the possible impact of the treatment on their product and will only issue an import permit once the applicant agrees to treatment.

Irradiated cat food – a special case

In 2008–09, there were reports of at least 87 cats in Australia fed a particular brand of imported pet food developing leucoencephalomyelopathy, a chronic and sometimes fatal neurological syndrome (Child et al. 2009). The product was a semi-moist, fish and poultry-based pet food with no cereal ingredient that had been irradiated at 50 kGy to address Australian biosecurity requirements. The same product, but not irradiated, was sold extensively overseas with no reports of the syndrome in cats associated with that product.

Signs were typically seen three to six months after first exposure to the pet food and the period the food was fed varied from about three weeks to more than six months. Post mortem examination of eight cats had shown lesions very similar to those of

cats fed irradiated pet food in Ireland (Cassidy et al. 2007; Caulfield et al. 2008; Caulfield et al. 2009) and in the United States (Duncan et al. 2009).

There is anecdotal evidence that in perhaps three cases, affected cats ate irradiated dog food produced by the same manufacturer (and apparently not cat food). A detailed toxicological screening of the pet food was unable to identify a causative agent.

An unconfirmed report of possible leucoencephalomyelopathy in several cats in Queensland, Australia in 2007, later emerged with a possible link to another irradiated cat food product that was subsequently removed from the market in late 2007.

The report by Cassidy et al. (2007) of cats exhibiting hind-limb ataxia associated with leucoencephalomyelopathy from a specific pathogen free (SPF) colony fed long term exclusively on gamma-irradiated food in Ireland provides evidence of a possible link to the particular gamma-irradiated diet used. Both SPF and conventional status cats were fed to appetite on the same commercial formula ration except that the ration fed to SPF cats had been gamma-irradiated at between 36.3 kGy and 47.3 kGy. No further cases occurred following the replacement of the irradiated diet with an equivalent pasteurised diet (Cassidy et al. 2007).

An experimental study (Caulfield et al. 2009) by the same group into the effect of long-term feeding of irradiated dry cat food was able to reproduce similar neurological lesions. Following feeding of a diet irradiated at either 25.7–38.1 kGy or 38.1–53.6 kGy, increasing numbers of cats presented with progressively severe ataxia from day 140. Analysis of the diet revealed a reduction in vitamin A levels of 43–48 per cent, although the reduced level was still within the maintenance levels for adult cats recommended by the Association of American Feed Control officials and the United States National Academy of Sciences' National Research Council. Levels of fat were unchanged, although peroxide levels were substantially elevated, leading the authors to initially conclude that free radical formation (due to the irradiation) and simultaneous reduction in vitamin A might play an important role in the pathogenesis of the disease. This study provided further evidence that some gamma-irradiated diets might cause a neurological syndrome in cats. However, it is not clearly demonstrated that the vitamin A deficiency or the increase of peroxide or a combination of these is the cause for these neurological effects associated with leucoencephalomyelopathy and require further investigation and research (EFSA Panel on Food Contact Materials 2011).

Researchers investigating multiple sclerosis (Duncan et al. 2009) reported that cats fed an irradiated diet during gestation developed a severe neurological disease, resulting in severe myelin degeneration. Cats returned to a normal diet recovered slowly within three to four months. Biochemical analysis of the food and tissues from affected cats showed no dietary deficiencies or toxic accumulations.

There is also an unconfirmed report that about 15 years ago, cats fed irradiated dry product in an SPF breeding colony in the United States developed a similar neurological condition on three separate occasions. The two pet food companies

involved apparently decided not to report the event in the literature (pers. comm. in Child 2009).

Based on a review of the above reports, in April 2009 the department concluded that the cases of severe, chronic leucoencephalomyelopathy in cats in Australia were most likely linked to the irradiation of either the pet food or its packaging, that the problem appeared to have been restricted to cats, and that the causative agent or induced nutritional deficiency associated with the pet food was unknown and unlikely to be determined. The department concluded that the use of gamma irradiation for pet food for cats could no longer be supported as a biosecurity treatment. To address the possible risk associated with cats being fed irradiated dog food, the department now requires, as a condition on the import permit, that imported irradiated dog food be appropriately labelled as not fit for consumption by cats.

Effect on other materials including packaging

Polymers (for example, plastics) are often used for packaging food and other products. They are large molecules made up of a 'backbone' of linked, repeated simple structural units typically connected by covalent chemical bonds. Exposure to irradiation may cause a breakage of this 'backbone'. Three types of reaction may result from this breakage:

a) a recombination of the structure that does not result in any damage

b) a cross-linking of the chains producing even longer chains and possibly a stronger product, or

c) chain scission where the broken chains are terminated, often by free radicals produced by the ionisation, producing a weaker product.

Other radiation-induced changes include colour and odour changes. The effect of radiation may be reduced by using radiation-resistant polymers or adding antioxidants to the polymer base (Hansen and Shaffer 2001).

Irradiation is used commercially for its ability to modify various materials by these mechanisms. For example, radiation cross-linked polyurethanes, polyvinyl chloride and fluoro-polymers are used in a range of insulating materials. Irradiated natural polymers have a variety of uses in agriculture, medicine and bioremediation. Doses of 15 kGy can be used for radiation vulcanisation of natural rubber and latex (Chmielewski and Haji-Saeid 2005).

The effect of irradiation on metals is generally very small at the doses typically used for sterilisation. The use of electron beam processing of product contained in metal containers may be impractical due to the lack of penetrating power, but gamma and X-rays are able to more readily pass through metal containers (Hansen and Shaffer 2001).

3 Sterilisation and bio-burden

Sterility assurance level and bio-burden

Sterilisation is often perceived as an absolute concept involving the destruction or inactivation of all microorganisms. In reality, sterility is a probability, not an absolute. It cannot be known whether all microorganisms have been inactivated as we may not be aware of the existence of some microbial species, and it is not possible to culture or test for all species. It is therefore necessary to employ a 'process' definition to sterilisation, expressing sterility as a probability.

SAL is a term used in microbiology to describe the acceptable probability of a single organism being viable after it has been subjected to the sterilisation process. Because absolute sterility cannot be ensured, SAL is used as a predetermined limit to the number of potentially infective organisms that would be tolerated in the product following treatment. The SAL, used in this context, is based on the intended use of a product and the risk determined to be acceptable. For example, medical device and pharmaceutical manufacturers design their sterilisation processes for a SAL to ensure that no more than 'one in a million' devices are non-sterile. Expressed differently, the desired SAL is usually set at 10^{-6} which provides an assurance that there is less than one chance in a million of contamination in any one unit. SAL is also used to describe the killing efficacy of a sterilisation process, where a very effective sterilisation process has a very high SAL (Lambert 2004).

The effectiveness of the sterilisation process is typically measured as the reduction in the titre of an organism in the product over a set period. Each log reduction (i.e. 10^{-1}) represents a 90 per cent reduction in microbial population. Hence, a process shown to achieve a '6 log reduction' (i.e. 10^{-6}) will theoretically reduce a population from a million viable organisms (10^{6}) to one viable organism.

In microbiology, it is impossible to prove that all organisms have been destroyed due to lack of sensitivity of testing and the impracticality to test for every possible species of organism. As measurement of inactivation is based on titre reduction, it cannot be established that the titre of a contaminating organism in a product is reduced to zero. For example, if the initial titre of the contaminating organism in a product is 1000 organisms per gram (i.e. 10^{3}/g), a three log reduction will reduce the titre to one organism per gram. However, a six log reduction would decrease the titre to one organism per kilogram (i.e. 10^{-3}/g).

SALs may also be used to describe the 'probability of a non-sterile unit' and may be expressed as PNSU in some literature. The required sterilisation dose (DS) depends on the initial microbiological contamination (i.e. bio-burden; N), the radiosensitivity of microorganism (D_{10}) and the assurance of sterility required (SAL) calculated as (Gazsó and Gyulai 2004):

$$DS = D_{10} (\log_{10}N - \log_{10}SAL) \quad [2]$$

According to Ponta (2005), 25 kGy was originally chosen as the DS for medical devices to achieve a SAL of 10^{-6} based on a bio-burden of 10^2 colony forming units of *Bacillus pumilus* (the most radio-resistant microorganism known at the time) which has a D_{10} of 3.1 kGy. Based on the formula [2] above;

$$DS = 3.1 \times (2 - (-6)) \simeq 25 \text{ kGy} \quad [3]$$

More irradiation-resistant microorganisms have since been reported and, although 25 kGy is still generally accepted an appropriate dose for most medical devices, authorities usually require a DS to be validated based on bio-burden level and D_{10} value for likely contaminants and the agreed SAL. A SAL of 10^{-6} is generally mandatory by convention.

A more detailed description for SAL, including its history, can be found in Mosley (2008).

Recommendations: application of bio-burden and SAL to dose rate

- The gamma irradiation dose to address animal biosecurity concerns should be based on the likely bio-burden in the product, before irradiation, of each viable pathogen of concern.
- SAL to address animal biosecurity concerns should be set at 10^{-6} for each pathogen of concern unless an alternative SAL is established, through a risk analysis, for the specific pathogen and product.
- The recommended SAL of 10^{-6} may be achieved by using a minimum gamma irradiation dose calculated by combining the likely bio-burden with the D_{10} for the pathogen of concern.
- It is generally assumed that tissues used in the manufacture of biological products such as vaccines would be derived from healthy sources and that in most circumstances, the bio-burden is close to zero.
- An instance where the bio-burden is anticipated to be higher than zero would be in a situation where infected tissue may be imported into a laboratory for further research. In these situations, the bio-burden in the infected tissue must be considered on a case-by-case basis and a higher dose may be recommended. For example, if the bio-burden is 10^3 plaque-forming units (PFUs), a nine log reduction in titre is required to achieve the SAL. If the D_{10} for the pathogen is 5 kGy, a dose of 45 kGy is required.

Dosimetry

Dosimetry systems are used to quantify the dose absorbed in a material. A primary dosimetry standard uses the rise in temperature of the product caused by the irradiation. One calorie (i.e. 4.18 joules) of heat per gram of water raises its temperature by 1^oC. As 1 gray = 1 joule/kilogram, 1 kGy raises the temperature of water by $1/4.18 = 0.239^oC$. Reference dosimeters are used at the irradiation facility and are usually calibrated against a primary standard. There are several types of dosimeters including those that measure the change in optical density in

radiosensitive dyes such as polymethyl methacrylate and radiochromic film (Hansen and Shaffer 2001; Miller 2005).

Validation of processing

Validation of sterilisation is essential to establish the conditions that perform irradiation sterilisation both properly and consistently. The biological effect of irradiation depends on the accumulated absorbed dose, regardless of the irradiation type, rate of delivery of the dose, or interruption in delivering the dose. Non-homogeneity of dose delivery to all parts of the treated material cannot be avoided although dose-mapping of specific loaded containers can be undertaken. The minimum dose received by any part of the treated material (Dmin) is necessary to calculate the irradiation time as Dmin has to be equal to or greater than the DS. Non-homogeneity can be quantified by Dmax/Dmin. Validation procedures require prior knowledge of pre-treatment bio-burden, DS and operational parameters to achieve that dose, and the loading pattern of the sterilising facility including the Dmin and Dmax positions in the irradiation container (Ponta 2005).

Approval to use irradiation as a biosecurity option to address exotic animal disease concerns should take into consideration product parameters relevant to the effectiveness of irradiation (for example, radiodensity of materials, homogeneity of product) environmental factors (for example, water and oxygen levels), and if necessary, the animal health status of the country of origin, other processing treatments and certification confidence.

Recommendations: validation

Validation of the irradiation process is necessary especially for non-homogenous products, radiodense materials or high biosecurity risk products (based on country and species of origin and end use). Where used as a routine treatment for a product, the treatment should be applied both properly and consistently each time.

4 Effect on microorganisms

Ionising radiation can cause either irreversible, irreparable damage leading to inactivation of the microorganism, or it can cause damage that can be repaired (sub-lethal). The potential for lethal damage is influenced by a range of environmental conditions such as chemicals, oxygen, temperature and water.

Gamma rays ionise atoms and molecules as they pass through matter. This ionisation may alter the molecular structure or spatial configuration of biologically active macromolecules. It also causes excitation and dissociation of water, leading to the formation of free radicals that in turn leads to a range of biochemical reactions and the homogenous distribution of free radicals. Damage to the nucleic acid would prevent replication of a microorganism. Damage to proteins and enzymes within the organism may be less critical as they can be replaced if the nucleic acid is undamaged. Indirect damage may also be caused by diffusible free radicals produced by the ionisation and subsequently interfering with structural or cellular functions such as the enzyme system, although both bacteria and viruses have been shown to display some self-protection against free radicals.

The damage or 'lesion' caused by ionisation to a macromolecule at or near it has been referred to as a 'hit', the most sensitive target being the nucleic acid. A lesion to the nucleic acid can be due to a single-strand break, a double-strand break (if a double-stranded nucleic acid), or a loss or alteration to one or more bases.

Although inactivation of microorganisms by ionising radiation usually follows an exponential curve, some organisms display a sigmoid inactivation curve with a brief 'shoulder' before the exponential inactivation commences. This 'shoulder' may be due to the repair mechanism of the organism with a proportion of the organisms requiring multiple 'hits' to be inactivated (Hansen and Shaffer 2001). Figure 2 provides an example of inactivation curves.

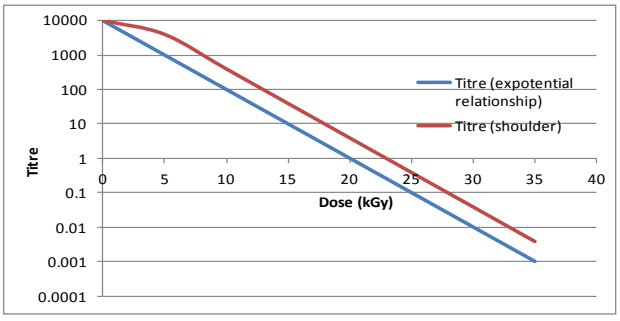

Figure 2. Example inactivation curves

As discussed previously, a sterilisation process can be defined in terms of bio-burden and the required SAL. However**,** the process can be complicated by other factors such as the physiological state of the organisms, the environmental conditions and even the strain of the agent of concern.

Many products can protect against the effect of irradiation while others may sensitise organisms to ionising irradiation. Sulfhydryls such as cystamine, cysteine and

glutathione are examples of protective agents whereas phosphate compounds tend to make organisms more sensitive to irradiation damage (Reid and Fairand 1998).

Targets irradiated in an aerobic environment generally show greater sensitivity compared to irradiation in an anaerobic environment. Heating followed by irradiation can have a greater inactivation effect than the additive rates of the individual treatments. This increased sensitivity to subsequent irradiation may be due to a reduction in the 'shoulder' of the inactivation curve. In contrast, irradiation followed by heating does not appear to be as effective as the former sequence (Fisher and Pflug 1977).

The water content of the microbial cell at irradiation also significantly affects its irradiation response, with increasing water content increasing sensitivity of microorganisms to irradiation. Oxygen has been shown to increase the sensitivity to irradiation of almost all types of organisms. Three kinds of irradiation damage to the organism have been proposed. First, damage independent of oxygen; second, immediate oxygen dependent damage due to interaction of oxygen with short-lived radicals produced during irradiation; and third, post-irradiation oxygen dependent damage due to interaction of oxygen with long-lived free radicals (Gazsó 2005). However, the same factors that increase the sensitivity of microorganisms to irradiation may also increase radiolytic changes in the product.

The radiation resistance of microorganisms can be either increased or decreased by altering the environment that exists during irradiation. Dose modifying factors are listed in Table 1.

Table 1. Factors that modify radiation resistance (modified from Hanson and Shaffer 2001)

Modifier	**Example**	**Effect on resistance**	**Conditions that influence modifier**
Atmosphere	Oxygen	Decrease	Reducing agents, protectors, anaerobiosis by microbial metabolism or by dose, catalase
Protectors	Sulfhydryl-containing compounds, reducing agents, alcohols, glycerol, dimethyl sulfoxide, proteins and carbohydrates	Increase	Oxygen, pH, and temperature
Temperature	Freezing Elevated	Increase Decrease	

Modifier	Example	Effect on resistance	Conditions that influence modifier
Water content of cell	Desiccation of cells	Increase—vegetative Decrease—spores or yeasts	Relative humidity, oxygen
Recovery technique	Incubation temperature, composition of medium, salts, diluents, oxygen	Variable	

Radiosensitivity of viruses

Radiation sensitivity is inversely proportional to the size and complexity of the organism. A large target is more sensitive to ionising radiation compared to a smaller one. Viruses have very small genomes (compared to bacteria and fungi) resulting in their higher resistance to irradiation. In general, viruses are less susceptible to ionising irradiation than bacteria, including bacterial spores, and require higher doses of irradiation (Farkas 2007).

The principal lesions induced by ionising radiation in intracellular nucleic acids are chemical damage to the purine and pyrimidine bases and to deoxyribose sugar, and a physiochemical damage resulting in a single-strand break or double-strand break. Single-stranded viruses are more sensitive to irradiation than are double-stranded viruses despite their smaller, simpler genome. Single-stranded viruses are homogenous with respect to their radio-sensitivity and inactivation does not require cumulative damage. Although strand breakage is an important cause of inactivation for single-stranded viruses, the combination of base damage and intra-strand cross-link formation is also important.

Although viruses have the theoretical ability to repair their genome, this is likely to be a rare occurrence. Although every virus conforms to ‘single hit’ kinetics, variation in response to irradiation—as seen with the ‘shoulder’ on some virus inactivation curves—reflects varying environments, sensitive and insensitive strains of virus present, clumping of virus particles, and/or the presence of protectors in the medium.

For double-stranded DNA (dsDNA), scission of the double-strand or simultaneous base-pair damage is necessary for effective inactivation. For irradiation to be most effective, it must damage either strand or one segment of the DNA that is critical to the operation of the virus. Besides direct damage to the DNA, alterations to the protein structures necessary for host attachment and for injection of DNA into the host may also be responsible, at least in part, for inactivation of DNA viruses by ionising radiation. Although viruses with large genomes may be more sensitive than those with small genomes (within each single or double-stranded group), resistance will vary considerably depending on a range of factors—in particular, organic material in the suspending substrate, oxygen concentration, pH during irradiation,

temperature and water activity (Gazsó 2005; Hilmy and Pandansari 2007; Reid and Fairand 1998).

The lethal effect of ionising radiation on viruses increases in the presence of oxygen. In the total absence of oxygen, radiation resistance increases by a factor of two to four. In dry conditions, the radiation resistance may increase by a factor of 8 to 17 (Farkas 2007).

Viruses irradiated in a liquid medium are more sensitive than either dried or frozen samples. Fully wet virus is most sensitive with resistance increasing with dehydration. The media used, especially its water content, and the possibility of both damp and dry populations of virus in the sample, should be considered in reviewing the effectiveness of irradiation.

Many viruses have D_{10} values exceeding 5 kGy. There is often considerable variation between published research results, possibly attributable to viral strain differences, environmental factors (for example, media, temperature and water content) and laboratory and titration method. There are also the occasional high, and probably anomalous, D_{10} results reported such as 13 kGy for foot-and-mouth disease virus (FMDV) when irradiated in the frozen state (Hilmy and Pandansari 2007), 21 kGy for porcine parvovirus (Willkommen et al. 1999), 25 kGy for bovine herpesvirus (Rojas et al. 2006) and 29 kGy for bovine parvovirus (Hermann et al. 2005).

Table 2 provides a summary of D_{10} values for a wide range of virus families. More detailed information is provided in Table 8, Appendix 3.

Table 2. Estimated gamma irradiation D_{10} values for virus families

Family	Virus structure	Presence of an envelope	Virion size (Daltons)	Diameter (nm) of the virion	D_{10}	
					Min	Max
Adenoviridae	dsDNA	No	150–180 x 10^6	70–90	3.50	5.91
Arenaviridae	NssRNA	Yes	Not available	(50–)110–130(–300)	2.00	3.20
Arteriviridae	ssRNA	Yes	Not available	60	11.10	12.50
Asfarviridae	dsDNA	Yes	Not available	175–215	<2.00	Unknown
Birnaviridae	dsRNA	No	55 x 10^6	60	6.20	10.00
Bunyaviridae	NssRNA	Yes	300–400 x 10^6	(80–)100–(–120)	<2.00	3.50

Family	Virus structure	Presence of an envelope	Virion size (Daltons)	Diameter (nm) of the virion	D_{10}	
					Min	Max
Caliciviridae	ssRNA	No	~15 x 10^6	35–39	0.01	3.3
Circoviridae	ssDNA	No	Not available	17–26.5	Not available	Not available
Coronoviridae	ssRNA	Yes	400 x 10^6	120–160	<2.00	<3.60
Filoviridae	NssRNA	Yes	382 x 10^6	~ 80	1.20	2.30
Flaviviridae	ssRNA	Yes	Vary depending on the genus (~60 x 10^6)	40–60	1.80	8.60
Herpesviridae	dsDNA	Yes	Not available	120–200	1.47	7.31
Orthomyxoviridae	NssRNA	Yes	250 x 10^6	80–120	0.70	7.08
Paramyxoviridae	NssRNA	Yes	500 x 10^6	(60–)150–200	0.65	5.20
Parvoviridae	ssDNA	No	5.5–6.2 x 10^6	18–22, or 20–26	4.00	21.05
Picornaviridae	ssRNA	No	8–9 x 10^6	(22–) 27–30	1.20	8.10
Polyomaviridae	dsDNA	No	10–13% of the virion by weight	40–55	3.90	12.00
Poxviridae	dsDNA	Yes	85–250 x 10^6 (160 x 10^6 to 200 x 10^6)	140–260 or 160–190	1.70	8.14
Reoviridae	dsRNA	No	120 x 10^6	60–80	1.00	10.5
Retroviridae	ssRNA-RT	Yes	2% of the virion by weight	80–100	1.31	10.6
Rhabdoviridae	NssRNA	Yes	300–1000 x 10^6	100–430	2.00	2.90
Togaviridae	ssRNA	Yes	52 x 10^6	70	3.87	10.20

Family Adenoviridae

The family Adenoviridae contains four genera: Atadenovirus, Aviadenovirus, Mastadenovirus and Siadenovirus; there are also unassigned viruses. The genus Aviadenovirus contains fowl adenovirus A, genus Atadenovirus contains ovine adenovirus D and genus Mastadenovirus contains human adenovirus C (ICTV 2009).

Virus structure

The genome is not segmented and contains a single molecule of linear dsDNA. Virus capsid is not enveloped, and is round with icosahedral symmetry.

Physicochemical and physical properties

Virions have a buoyant density in cesium chloride (CsCl) of 1.32–1.35 g cm^{-3}. Under *in vitro* conditions, virions are stable when stored at –20°C and in acid environment of pH 5–6. Virions are not sensitive to treatment with lipid solvents (Büchen-Osmond 2008).

Viruses

Adenovirus 3, 5 and 12

Sullivan et al. (1971) reported that adenoviruses 3, 5 and 12 required 4.9, 4.4 and 4.6 kGy respectively for one log reduction in Eagle's minimal essential medium (MEM) plus two per cent foetal bovine serum (FBS). This study was based on 103 observations and reported a range of D_{10} values for adenovirus 2 of 3.8 to 4.6 kGy. Although the species affected by this adenovirus was not specified, the data is still valuable as it is applicable to all adenoviruses.

Avian adenovirus (Genus Aviadenovirus)

Thomas et al. (1981) reported 4.5 kGy for one log reduction for avian adenovirus in cell culture medium.

Canine adenovirus

Sofer (2003) reported a D_{10} value of 3.5 kGy for canine adenovirus.

Invitrogen (2003) reported D_{10} values ranging from 5.17–5.9 kGy when irradiated in FBS.

Conclusion

Based on the above studies, the D_{10} range for viruses in the Adenoviridae family is estimated to be 3.5 to 5.9 kGy.

Family Arenaviridae

The family Arenaviridae contains only one genus: Arenavirus (ICTV 2009).

Virus structure

The genome is segmented and consists of two segments of linear, negative-sense to ambisense, single-stranded ribonucleic acid (ssRNA). Virions consist of an envelope and are spherical to pleomorphic.

Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.19–1.2 g cm^{-3}, in sucrose of 1.17–1.18 g cm^{-3}, and in amidotrizoate compounds of 1.14 g cm^{-3}. Under *in vitro*

conditions virions are stable when stored at –70°C, and sensitive to an acid environment of pH 5.5 and below, and an alkaline environment of pH 8.5 and above. Virions are sensitive to treatment with organic solvents (Büchen-Osmond 2008).

Viruses

Lassa virus (Genus Arenavirus)

Elliott et al. (1982) reported a D_{10} value of 2.0 kGy at 4°C and 3.1 kGy at –60°C in either phosphate-buffered saline (PBS)/bovine serum albumin (BSA) or human serum.

Conclusion

Based on the above study, the D_{10} range for viruses in the Arenaviridae family is estimated to be 2.0–3.2 kGy. This was the only study found to be applicable to the Arenaviridae family. In the absence of further data, the use of a higher D_{10} value may be justified when determining an appropriate minimum irradiation dose to address viruses in the Arenaviridae family.

Family Arterioviridae

The family Arterioviridae contains only one genus: Arterivirus. The type species of the genus is equine arteritis virus.

Virus structure

Virions consist of an envelope and a nucleocapsid. Virus capsid is enveloped. The genome is not segmented and contains a single molecule of linear positive-sense ssRNA.

Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.17–1.2 g cm^{-3} and in sucrose, of 1.13–1.17 g cm^{-3}.

Viruses

Porcine reproductive and respiratory syndrome virus

Purtle et al. (2009) reported that porcine reproductive and respiratory syndrome virus titres spiked into glucose were negative at doses above 22 kGy at pilot scale studies. Reductions of 4.83 and 5.0 log were shown when porcine reproductive and respiratory syndrome virus spiked into glucose were irradiated with 43–54 kGy in production scale studies.

Conclusion

Based on the above studies, the D_{10} range for viruses in the Arterioviridae family is estimated to be 11.1–12.5 kGy when irradiated in a dry environment. The estimated D_{10} range is higher than that estimated for many other ssRNA viruses in a moist environment and it is possible that the higher irradiation doses were required as the virus was irradiated in a dry environment. This was the only study found to be applicable to the Ateriovirdae family. In the absence of further data, a D_{10} value for viruses in family Ateriovirdae cannot be estimated and the current standard treatment of 50kGy should be used.

Family Asfarviridae

The family Asfarviridae contains only one genus: Asfarvirus. The type species in the Asfarvirus genus is African swine fever virus.

Virus structure

The genome is not segmented and contains a single molecule of linear dsDNA. Virus capsid is enveloped. Virions are spherical.

Physicochemical and physical properties

Capsid is enveloped, and has a buoyant density in CsCl of 1.19–1.24 g cm^{-3}.

Viruses

African swine fever virus (Genus Asfarvirus)

McVicar (1982) reported that 20 kGy inactivates African swine fever virus in pig lymph node, spleen and tonsil. Thomas (1981) reported <2.0 kGy for one log reduction in whole pig blood.

Conclusion

Based on the above study, the D_{10} range for viruses in the Asfarviridae family is estimated to be less than 2.0 kGy.

This was the only study found to be applicable to the Asfarviridae family. In the absence of further data, the use of a higher D_{10} value may be justified when determining an appropriate minimum irradiation dose to address viruses in the Asfarviridae family.

Family Birnaviridae

The family Birnaviridae contains three genera: Aquabirnavirus, Avibirnavirus and Entomobirnavirus. In the Aquabirnavirus genus, infectious pancreatic necrosis virus is the type species and in the Avibirnavirus genus, infectious bursal disease virus is the type species.

Virus structure

The genome is segmented and consists of two segments of linear, double-stranded RNA (dsRNA). Virions are not enveloped.

Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.33 g cm^{-3} or 1.3 g cm^{-3}. Under *in vitro* conditions, virions are stable in acid environments of pH 3–7 and stable in alkaline environments of pH 7–9. Virions are not sensitive to treatment with heat (60^{o}C for 60 minutes) or ether (and one per cent SDS at 20^{o}C, pH 7.5 for 20 minutes).

Viruses

Infectious bursal disease (Genus Avibirnavirus)

Jackwood et al. (2007) reported that titres of the three classic infectious bursal disease virus vaccine strains were reduced between 1.6–2.0 logs after the 10 kGy exposure (D_{10} value of 5–6.25); however, these viruses remained viable after this treatment.

Infectious pancreatic necrosis virus (Genus Aquabirnavirus)

Ahne (1982) reported a D_{10} of 10 kGy gamma irradiation for infectious pancreatic necrosis virus. This was the only study found to be applicable to the family Birnaviridae.

Conclusion

Based on the above studies, the D_{10} range for viruses in the family Birnaviridae is estimated to be up to 10 kGy. However, in the absence of further data, the use of a D_{10} value of 10 kGy may be justified when determining an appropriate minimum irradiation dose to address viruses in the family Birnaviridae.

Family Bunyaviridae

The family Bunyaviridae contains five genera: Hantavirus, Nairovirus, Orthobunyavirus, Phlebovirus and Tospovirus. Rift Valley fever virus is the type species for the Phlebovirus genus.

Virus structure

The genome consists of three segments of negative-sense and ambisense ssRNA. Virions consist of an envelope and are spherical to pleomorphic.

Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.2–1.21 g cm^{-3} and in sucrose of 1.16–1.18 g cm^{-3}. Virions are sensitive to treatment with detergents, formaldehyde, and heat.

Viruses

Akabane virus (Genus Orthobunyavirus)

Thomas et al. (1981) reported <2.0 kGy for one log reduction of Akabane virus in cell culture medium. House et al. (1990) reported one log reduction of Akabane virus at 2.5 kGy in bovine serum at –68°C using a tissue culture infective dose (TCID) assay.

Aino virus (Genus Orthobunyavirus)

House et al. (1990) reported one log reduction of Aino virus at 3.5 kGy in bovine serum at –68°C using a TCID assay.

Conclusion

Based on the above studies, the D_{10} for viruses in the family Bunyaviridae is estimated to be up to 3.5 kGy.

Family Caliciviridae

The family Caliciviridae contains four genera: Lagovirus, Norovirus, Sapovirus and Vesivirus. Enteric feline calicivirus and respiratory feline calicivirus belong to the genus Vesivirus.

Virus structure

The genome is not segmented and contains a single molecule of linear positive-sense ssRNA.

Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.33–1.4 g cm^{-3}. Under *in vitro* conditions virions are stable in acid environments of pH 4–5. Virions are not stable at raised temperature in the presence of a high concentration of Mg^{2+}. In some strains, virions are sensitive to treatment with trypsin and in other strains, the infectivity is enhanced after treatment with trypsin. Some strains are not sensitive to treatment with chloroform, mild detergents or ether.

Viruses

Murine norovirus (Genus Norovirus)

Murine norovirus 1 (MNV-1) is often used in inactivation studes as a human norovirus surrogate. Feng et al. (2011) observed a 1.7 to 2.4-log virus reduction of MNV-1 virus in fresh produce at the dose of 5.6 kGy. This equates to a D_{10} of 2.3 to 3.3 kGy.

Canine and feline caliciviruses (Genus Vesivirus)

De Roda Husman et al. (2004) reported that a 3-log virus reduction was observed in low-protein-content solution at 0.5 kGy gamma irradiation for feline calicivirus (FeCV) and 0.3 kGy for canine calicivirus (CaCV). This equates a D_{10} of 0.17 kGy for FeCV and 0.10 kGy for CaCV. These results are very low compared to other viruses in general including murine norovirus in the same family.

Conclusion

The literature on inactivation of caliciviruses by gamma radiation is not extensive. Based on the limited studies available, the D_{10} recommended to use for viruses in the Caliciviridae family, including those viruses in the Vesivirus genus, is 3.3 kGy.

Family Circoviridae

The family Circoviridae contains two genera: Circovirus and Gyrovirus. Porcine circovirus 1 is the type species in the genus Circovirus and chicken anaemia virus is the type species in the genus Gyrovirus.

Virus structure

The genome is monomeric, not segmented and contains a single molecule of circular, negative-sense, or ambisense, single-stranded DNA (ssDNA) that forms a covalently closed circle, and the virion is non-enveloped. Virion diameters for chicken anaemia virus, porcine circovirus and beak and feather disease virus are 19.1–26.5 nm, 17–20.7 nm, 12–20.7 nm respectively.

Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.33–1.37 g cm^{-3} (Fauquet et al. 2005).

Viruses

Porcine circovirus (Genus Circovirus)

There is very little data available on the radiosensitivity of circoviruses although, given their very small size, they are expected to be considerably resistant. According to Plasvic (2001), 45 kGy may not inactivate virus in FBS.

Conclusion

Given the lack of relevant inactivation studies, the D_{10} range for viruses in the Circovirus family cannot be estimated and an irradiation dose of 50 kGy should continue to be used.

Family Coronaviridae

The family Coronaviridae contains two genera: Coronavirus and Torovirus. Infectious bronchitis virus is the type species in the genus Coronavirus and equine torovirus is the type species in the genus Torovirus.

Virus structure

Coronaviruses are ssRNA positive-strand viruses that are enveloped. Virions are spherical to pleomorphic, or kidney-shaped, or rod-shaped.

Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.23–1.24 g cm^{-3} and in sucrose of 1.15–1.19 g cm^{-3}. Under *in vitro* conditions some viruses are stable in acid environment of pH 3. Virions are relatively stable in the presence of Mg^{2+} and they are sensitive to treatment with non-ionic detergents, formaldehyde, heat, lipid solvents and oxidizing agents (Fauquet et al. 2005).

Viruses

Transmissible gastroenteritis (Genus Coronavirus)

Thomas et al. (1981) reported 2.0 kGy for one log reduction in cell culture medium. Simon et al. (1983) reported 20 kGy inactivated six logs of virus in culture media and 5.5 logs in liquid manure. As this study did not examine the effect of other doses on transmissible gastroenteritis virus, it can only be estimated that its D_{10} in cell culture media is less than 3.1 kGy and for virus in liquid manure, less than 3.6 kGy.

Conclusion

Based on the available studies, the D_{10} range for viruses in the Coronaviridae family is estimated to be up to 3.6 kGy.

Family Filoviridae

The family Filoviridae contains two genera: Ebolavirus (formerly known as ‘Ebola-like viruses’) and Marburgvirus (formerly known as ‘Marburg-like viruses’).

Virus structure

Filoviruses contain negative-sense ssRNA (NssRNA) and are enveloped. Virions are bacilliform and filamentous with a diameter of ~80 nm.

Physicochemical and physical properties

Buoyant density of virions in potassium tarterate is 1.14 g cm^{-3}, infectivity of marburgviruses and ebolaviruses is stable at less than 20°C, but drastically reduced within 30 minutes at 60°C. Virus infectivity is sensitive to quaternary ammonium salt, hypochlorite and phenolic disinfectants, formaldehyde, beta propiolactone, and gamma and ultraviolet (UV) radiation (Fauquet et al. 2005).

Viruses

Ebola (Genus Ebolavirus)

Elliot et al. (1982) reported a D_{10} value for Ebola virus of 1.5 kGy at 4°C and 2.15 kGy at –60°C in either PBS/BSA or human serum. Lupton et al. (1981) reported a D_{10} value of 2.3 kGy for Ebola virus.

Marburg (Genus Marburgvirus)

For Marburg virus, Elliott et al. (1982) reported a D_{10} value of 1.2 kGy at 4°C and 2.1 kGy at –60°C in either PBS/BSA or human serum.

Conclusion

Based on the available studies, the D_{10} for viruses in the family Filoviridae is estimated up to 2.3 kGy.

Family Flaviridae

The family Flaviviridae contains genera Flavivirus, Hepacivirus and Pestivirus. Two distinct groups of viruses have also been assigned tentatively to the family Flaviviridae. Yellow fever virus, bovine viral diarrhoea virus (BVDV) 1 and hepatitis C virus type species are from the genera Flavivirus, Pestivirus and Hepacivirus, respectively.

Virus structure

Virions consist of an envelope and a nucleocapsid. The genome is not segmented and contains a single molecule of linear positive-sense ssRNA. Virus capsid is enveloped. Virions are spherical to pleomorphic.

Physicochemical and physical properties

Viruses have a buoyant density in CsCl of 1.07–1.24 g cm^{-3} and in sucrose of 1.1–1.23 g cm^{-3} (Büchen-Osmond 1995). Under *in vitro* conditions virions are stable in alkaline environment of pH 8. Virions are sensitive to treatment with heat, organic solvents, and detergents. The buoyant density of flaviviruses is about 190 g cm^{-3}, for the hepaciviruses it is about 1.16 g cm^{-3} and for pestiviruses it is about 1.10–1.15 g cm^{-3} (Fauquet et al. 2005).

Viruses

Yellow fever virus (Genus Flavivirus)

A study into the effect on irradiation on coagulation proteins reported an inactivation rate of 0.297 $\log_{10}$ $TCID_{50}$ doses/ml/kGy for yellow fever virus which is estimated to be a D_{10} of 3.37 kGy (Kitchen et al. 1989).

St Louis encephalitis (Genus Flavivirus)

A D_{10} value of 5.81 kGy for St Louis encephalitis virus in brain tissue and 6.2 kGy for crude virus has been reported by Jordan (1956).

West Nile virus (Genus Flavivirus)

Neidrig (1999) irradiated West Nile virus supernatants heated to 56°C for one hour and with 30 kGy. Infectivity was excluded by re-inoculation in *in vitro* cell cultures, with three subsequent passages.

Bovine viral diarrhoea virus (Genus Pestivirus)

There is complete inactivation of BVDV in the presence of factor VIII, fibrinogen and α-1 proteinase inhibitor by 20–30 kGy. Inactivation was less effective in freeze dried immunoglobulin (Miekka et al. 1998). Willkommen (1999) reported a 6.1 log reduction of BVDV in bovine serum by 25 kGy and a four log reduction by 20 kGy in equine serum, giving D_{10} values of 4.1 and 5 kGy respectively.

Pruss (2001) reported 18 kGy for a six log reduction in frozen bone, equating to a D_{10} of 3 kGy. He also reported D_{10} values ranging from <5.2 to <6.4 for BVDV in contaminated bone diaphyses.

Pruess (1997) reported D_{10} values for BVDV of 4.9 kGy in FBS and 2.5 kGy in liquid serum using e-beam radiation.

Thomas (1981) reported a D_{10} of up to 2.0 kGy for virus in cell culture medium.

Meikka (2003) reported a D_{37} of 3.0 kGy for virus in 25 per cent solution human albumin, equating to a D_{10} of 6.8 kGy. Hermann (2005) reported that a 6.1 log reduction was achieved with 35 kGy, giving a D_{10} of 5.7 kGy. This study also looked at the effect of UV treatment (eight log reduction by 254 nm UV-C) and combined treatment (14.1 log reduction).

Simon (1983) reported that 5.5 log of virus was completely inactivated by 20 kGy in culture media. As the effect of other doses was not examined, it can only be estimated that the D_{10} is less than or equal to 3.6 kGy. This study also reported that 6.5 log was inactivated by 20 kGy in liquid manure, giving a $D_{10} \leq 3.1$ kGy.

Purtle et al. (2006b) reported a 4.8 (production scale) to 5.8 (pilot scale) log reduction using 25–35 kGy. D_{10} = 5.2–7.3 (production scale) and D_{10} = 4.3–6.0 (pilot scale).

Hanson et al. (1993) reported that a 10 log reduction of a frozen sample required a dose of 48 kGy (D_{10} = 4.8).

Reid (1998) performed inactivation experiments on BVDV with albumin, factor VIII and fibrinogen as the media. The estimated D_{10} values for BVDV in albumin (8.3 kGy) and in fibrinogen (8.6 kGy) were similar yet substantially higher than for virus in factor VIII (5.5 kGy). A more substantial difference between factor VIII and the other blood proteins was not seen in the same study with PPV.

Invitrogen (2003) reported that more than 6.8 reduction using 25 and 35 kGy in FBS. The virus was inactivated to levels below limit of detection (0.5 $TCID_{50}$/mL) when irradiated with 45 kGy.

Classical swine fever virus (Genus Pestivirus)

House (1990) reported 5.5 kGy for a one log reduction of classical swine fever virus in bovine serum at –68^{o}C using a TCID assay. Groomsman et al. (1977) reported a D_{10} of 1.8 kGy for classical swine fever (Brescia strain) virus. Richmond (1981) also reported preliminary data from trials then being undertaken anticipating a D_{10} of 8.6 kGy for classical swine fever virus although this has not been published.

Conclusion

Based on the available studies, the D_{10} range for viruses in the family Flaviviridae is estimated to be between 1.80 and 8.6 kGy.

Family Herpesviridae

The family Herpesviridae contains three subfamilies (Alphaherpesvirinae, Betaherpesvirinae and Gammaherpesvirinae) and one unassigned subfamily.

The subfamily Alphaherpesvirinae contains four genera: Iltovirus, Mardivirus Simplexvirus and Varicellovirus; the subfamily Betaherpesvirinae contains three genera: Cytomegalovirus, Muromegalovirus and Rosealovirus; and the subfamily Gammaherpesvirinae contains two genera: Lymphocryptovirus and Rhadinovirus. The unassigned subfamily contains one genus: Ictalurivirus.

Virus structure

Virions are enveloped, spherical to pleomorphic, and the genome contains dsDNA.

Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.22–1.28 g cm^{-3}. Stability of different herpesviruses varies considerably, but they are generally unstable to dessication and pH. Infectivity is destroyed by detergents and lipid solvents (Fauquet et al. 2005).

Viruses

Porcine herpesvirus 1 (Aujeszky's/pseudorabies)

Pruss et al. (2001) reported 5.3 kGy was needed for one log titre reduction of frozen virus suspensions in plastic tubes and <6.5 to <7.0 kGy in bone (contaminated diaphyses).

According to Iordano et al. (1979), virus was inactivated by 16 kGy in 50 per cent Hank's balanced saline solution, 50 per cent Eagle's MEM and a supplement of 10 per cent normal calf serum; and by 15 kGy in pig skin and greasy wool (Iordanov et al. 1979).

Thomas et al. (1981) reported <2.0 kGy for one log reduction in cell culture medium and Thomas et al. (1982) reported that no virus was recovered from cell culture medium and cell debris in liquid pig faeces, although the results were undetermined.

Sun et al. (1978) reported that approximately eight log reduction was achieved with 35.6 kGy, achieving a D_{10} of 4.56 kGy.

Simon et al. (1983) reported that seven log of virus in culture media was inactivated by 20 kGy achieving a D_{10} of <2.86 kGy. The study also reported 6.5 log in liquid manure was inactivated by 20 kGy achieving a D_{10} of <3.1 kGy.

Richmond (1981) stated a D_{10} of 1.47 kGy for virus in suspension and 4.67 kGy for lyophilised virus.

Herpes simplex virus

Similar results were obtained for another herpesvirus: herpes simplex virus (HSV). Smolko and Lombardo (2005) reported D_{37} values of 1.12 to 1.34 kGy, which are

equivalent to D_{10} values of 2.58 to 3.08 kGy. A study into the radiation effect on the survivability of HSV reported D_{37} values of 1.2 kGy (D_{10} = 2.76 kGy) for HSV 1 Theta, 1.6 kGy (D_{10} = 3.68 kGy) for HSV 1 Ang, 1.0 kGy (D_{10} = 2.3 kGy) for HSV 1 Kos, and 1.6 kGy (D_{10} = 3.68 kGy) for HSV 1 Muller (Rösen et al. 1987). A slightly higher result of 4.3 kGy for the D_{10} of HSV has been reported (Sullivan et al. 1971). A study into the effect of irradiation on coagulation proteins reported an inactivation rate of 0.284 $\log_{10}$ $TCID_{50}$ doses/mL/kGy for HSV-1 which is estimated to be a D_{10} value of 3.52 kGy (Kitchen et al. 1989).

Infectious bovine rhinotracheitis

Infectious bovine rhinotracheitis (IBR) virus (bovine herpesvirus 1) was reported by Thomas et al. (1981) to have a D_{10} value of <2 kGy in cell culture medium. Purtle et al. (2006b) reported an inactivation level of 4–6 logs with a dose of 25–30 kGy. SAFC Biosciences (2006) reported an inactivation level of five logs at 25–35 kGy (D_{10} = 5–7). Hanson and Wilkinson (1993) reported that a 10 log reduction required a dose of 31 kGy (D_{10} =3.1).

Willkommen (1999) reported a seven log reduction of virus in FCS by 20 kGy (equivalent to a D_{10} value of 2.86 kGy).

Hermann (2005) reported a 7.1 log reduction achieved by 35 kGy which was equivalent to a D_{10} of 4.92 kGy. This study also looked at the effect of UV inactivation (six log reduction by 254 nm UV-C) and combined treatment (13.1 log reduction).

Degiorgi (1999) reported a D_{10} of 4.72 kGy at a temperature of –78^{o}C before removal of cell debris and 7.31 kGy after cell debris removal.

Invitrogen (2003) reported that a dose of 25 kGy and 35 kGy inactivated 6.0–7.82 logs and more than 7.82 respectively.

No information was found regarding bovine herpesvirus 2 and 4; however, the information for other viruses in the Herpesviridae family could potentially be extrapolated to these two viruses.

Turkey herpesvirus

A study into the effect of irradiation on coagulation proteins reported an inactivation rate of 0.196 $\log_{10}$ $TCID_{50}$ doses/mL/kGy for turkey herpes virus which is estimated to be a D_{10} of 5.10 kGy (Kitchen et al. 1989).

Bovine herpesvirus 1

Purtle (2006b) showed that a dose of 25–30 kGy reduced viral load by 5.31 logs in pilot scale inactivation (in FBS) and more than 4.69 in production scale inactivation (in donor horse serum) trials.

Conclusion

Based on the available studies, the D_{10} range for viruses in the Herpesviridae family is estimated to be between <2 and 7.31 kGy.

Family Orthomyxoviridae

The family Orthomyxoviridae contains five genera: Influenza virus A, Influenza virus B, Influenza virus C, Isavirus and Thogotovirus.

Virus structure

Virions consist of an envelope. Virions are spherical to pleomorphic, but filamentous forms do occur.

Physicochemical and physical properties

Virions have a buoyant density in sucrose of 1.17–1.2 g cm^{-3} genome consists of NssRNA, are enveloped, spherical or pleomorphic. Virion buoyant density in aqueous sucrose is 1.19 g cm^{-3}. Virions are sensitive to heat, non-ionic detergents, formaldehyde, irradiation and oxidising agents.

Viruses

Influenza virus A (Genus Influenzavirus A)

Sullivan (1971) reported 4.6 to 4.9 kGy was required for one log reduction in Eagle's MEM plus 2 per cent FBS and 1 kGy for a one log reduction in water. The potential for substantially different results based on the titration method was demonstrated by Lowy in which a D_{10} for PR8/H1N1 and X31/H3N2 of 2.82 kGy and 2.46 kGy respectively using PFU assays and 5.77 and 7.08 kGy respectively using $TCID_{50}$ assays (Lowy et al. 2001).

Thomas et al. (1982) reported a D_{10} of 0.7 kGy for avian influenza in liquid chicken faeces.

Conclusion

Based on the available studies, the D_{10} for viruses in the family Orthomyxoviridae is estimated to be up to 7.08 kGy.

Family Paramyxoviridae

The family Paramyxoviridae includes the subfamilies Paramyxovirinae and Pneumovirinae. The subfamily Paramyxovirinae has five genera: Avulavirus, Henipavirus, Morbillivirus, Respirovirus and Rubulavirus. The subfamily Pneumovirinae contains two genera: Metapneumovirus and Pneumovirus.

Virus structure

Genome is NssRNA. Virion is enveloped, pleomorphic, but usually spherical in shape, although filamentous and other forms are common.

Physicochemical and physical properties

Viral buoyant density in sucrose is 1.18–1.20 g cm^{-3}. Virions are very sensitive to heat, lipid solvents, ionic and non-ionic detergents, formaldehyde and oxidising agents.

Viruses

Newcastle disease (Genus Avulavirus)

Sullivan et al. (1971) reported 5.2 kGy for one log reduction in Eagle's MEM plus 2 per cent FBS. Thomas et al. (1981) reported a D_{10} of 2 kGy.

DiGioia et al. (1970) reported that 12.5 kGy achieved 7.5 log reduction in virus infectivity at 2.2°C giving an estimated D_{10} of 1.71 kGy. The haemagglutinating property of virus was not affected and there was a marked increase in radiosensitivity at temperatures above 49°C.

Brodorotti (1978), as quoted by Richmond (1981), reported a D_{10} of 2.58 kGy for virus in suspension.

Measles (Genus Morbillivirus)

A three log reduction at 25°C by approximately 0.21 x 10^6 roentgen equivalent physical (rep) of gamma irradiation has been reported (Musser et al. 1960). This equates to a D_{10} of 0.65 kGy. A study into the effect of irradiation on coagulation proteins reported an inactivation rate of 0.244 $\log_{10}$ $TCID_{50}$ doses/mL/kGy for measles virus which is estimated to be a D_{10} of 4.10 kGy (Kitchen et al. 1989).

Rinderpest virus (Genus Morbillivirus)

Saliki (1993) reported that the RBOK vaccine strain of rinderpest virus was reduced from 6.5 $\log_{10}$ $TCID_{50}$ to <1.0 $\log_{10}$ $TCID_{50}$ by 10, 20, 30, 40, 50, and 60 kGy at -70°C. This equates to a D_{10} of 1.8 kGy.

Bovine parainfluenza 3 (Genus Respirovirus)

Willkommen (1999) reported a 6.7 log reduction of virus in serum by 25 kGy, equating to a D_{10} of 3.75 kGy. Hermann et al. (2005) reported a 7.5 log reduction achieved by 35 kGy equating to a D_{10} of 4.67 kGy. This study also looked at the effect of the UV treatment (seven log reduction by 254 nm UV-C) and combined treatment (14.5 log reduction).

Conclusion

Based on the available studies, the D_{10} for viruses in the family Paramyxoviridae is estimated to be up to 5.20 kGy.

Family Parvoviridae

The family Parvoviridae contains two subfamilies: Densovirinae and Parvovirinae. The subfamily Parvovirinae has five genera: Amdovirus, Bocavirus, Dependovirus, Erythrovirus and Parvovirus. The subfamily Densovirinae contains four genera: Brevidensovirus, Densovirus, Iteravirus and Pefudensovirus, as well as some unassigned viruses.

Virus structure

Virions consist of a capsid. Virus capsid is not enveloped, and is round with icosahedral symmetry. The nucleocapsid is isometric and has a diameter of 18–22 nm or 20–26 nm. The genome is not segmented and contains a single molecule of linear negative-sense, or negative-sense and positive-sense ssDNA.

Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.38–1.45 g cm^{-3}.

Viruses

Minute virus of mice (Genus Parvovirus)

House et al. (1990) reported 10.7 kGy for one log reduction of minute virus of mice in bovine serum at –68°C using a TCID assay. This equates to a D_{10} of 10.7 kGy.

Feline panleucopenia (Genus Parvovirus)

A study into the effect on irradiation on coagulation proteins reported an inactivation rate of 0.131 log_{10} $TCID_{50}$ doses/mL/kGy for feline panleucopenia (feline infectious enteritis) which is estimated to be a D_{10} of 7.63 kGy (Kitchen et al. 1989).

Porcine parvovirus (Genus Parvovirus)

A four log reduction of porcine parvovirus (PPV) in the presence of fibrinogen, factor VIII, α-1 proteinase inhibitor and IgG was inactivated by 23, 28, 30 and 36 kGy respectively, giving a D_{10} ranging from 5.75 to 9.0 kGy (Miekka et al. 1998). Another study demonstrated a D_{37} of 2.3 to 3.0 kGy for PPV in 25 per cent solution of human albumin, equating to a D_{10} of 5.2–6.85 kGy (Miekka et al. 2003).

Willkommen (1999) reported that PPV in serum was not inactivated by 40 kGy, only achieving a 1.9 log reduction. Preuss et al. (Preuss et al. 1997) reported a D_{10} of 11.8 kGy in frozen bovine serum and 7.7 kGy in liquid serum using e-beam radiation.

Grieb (2005) reported that 50 kGy resulted in a 5.2 log reduction of the virus in pulverized bone, equating to a D_{10} of 9.6 kGy. Thomas et al. (1981) reported a D_{10} of 4.0 kGy in cell culture fluid.

Reid (1998) performed inactivation experiments on PPV with albumin, factor VIII and fibrinogen as the medium. The estimated D_{10} value for PPV in albumin (6.7 kGy), factor VIII (6.3 kGy) and fibrinogen (7.0 kGy) were similar.

Invitrogen (2003) reported that a dose of 25 kGy and 35 kGy effectively destroyed >7.09 logs of $TCID_{50}$/mL in FBS (below the limit of detection).

Bovine parvovirus (Genus Dependovirus)

Pruss et al. (2002) reported that at least 34 kGy is needed for four logs titre reduction of bovine parvovirus in bone at –30±5°C. From the study, D_{10} is estimated to be 7.4–10.1 kGy. Pruss et al. (2002) also reported a D_{10} of 7.3 kGy for frozen virus suspensions in plastic tubes.

Hermann et al. (2005) reported that 1.8 log reduction was achieved by 35 kGy, equating to a D_{10} of 19.45 kGy. The study also looked at the effect of UV treatment (eight log reduction by 254 nm UV-C) and combined treatment (9.8 log reduction).

Conclusion

Based on the available studies, the D_{10} range for viruses in the family Parvoviridae is estimated to be 4.0–21.05 kGy. The studies also show that more than 62.5 per cent trials required D_{10} of over 7 kGy. These findings suggest that parvoviruses may require a higher dose than other viruses for inactivation. However, there are no

animal parvoviruses known to be exotic to Australia and therefore the high radioresistance of parvovirus is less significant than for exotic pathogens.

Family Picornaviridae

The family Picornaviridae contains 10 genera: Aphthovirus, Cardiovirus, Enterovirus, Erbovirus, Hepatovirus, Kobuvirus, Parechovirus, Rhinovirus and Teschovirus.

Virus structure

Virions consist of a capsid. Virus capsid is not enveloped, round with icosahedral symmetry. The capsid is isometric and has a diameter of 27–30 nm. Capsids appear round. The genome is not segmented and contains a single molecule of linear positive-sense ssRNA.

Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.33–1.45 g cm^{-3}. Under *in vitro* conditions virions are relatively stable, or not stable in an acid environment of pH 6. Virions are relatively stable. Virions are not sensitive to treatment with chloroform, ether, and non-ionic detergents.

Viruses

Foot-and-mouth disease (Genus Aphtovirus)

The World Organisation for Animal Health *Terrestrial Animal Health Code* (2011) recommends a gamma irradiation dose of at least 20 kGy at room temperature (20^{o}C or higher) to address foot-and-mouth disease (FMD) concerns for skins and game trophies (OIE 2011).

Dekker (1998) reported that both 40 and 60 kGy inactivated types A, O, C and Asia of FMDV (air-dried virus) to levels below detectable levels. Type O, C and Asia virus were also inactivated by 30 kGy to levels below detection (achieving 6.4 to 7.0 log reduction) whereas 30 kGy only achieved a 4.6 log reduction of type A virus. This equates to a D_{10} ≤4.7 kGy for types O, C and Asia and at least 6.5 kGy for type A.

Groneman et al. (1977) and others reported a D_{10} of 4.3 to 4.7 for FMDV in culture fluid and 6.5 kGy for FMDV in sewage sludge demonstrating that the sludge had a protective effect against irradiation for FMDV (Dekker 1998; Groneman et al. 1977; Richmond 1981).

Baldelli (1965) recommended 17.6 kGy for disinfection of blood from infected animals. Massa (1966) as quoted by pers.comm. Richmond to Callis, Plum Island Animal Disease Centre, US (1981), reported a D_{10} of 4.6 kGy for type O, 4.8 kGy for type A, 4.8 kGy for type C, 5.0 kGy for type C in liquid state and 6.7 kGy for type C in a dry state (Dekker 1998; Richmond 1981). Polatnick and Bachrach (1968) reported that 40 kGy was required for an eight log reduction in type A, strain 119 FMDV and that the growth media and cellular products protect the virus. This equates to a D_{10} of 5 kGy.

House et al. (1990) reported that 5.3 kGy was required for a one log reduction of virus in bovine serum at –68^{o}C using a TCID assay. Simon et al. (1983) reported that seven log of type C FMDV (extracellular virus and cell associated virus) was

inactivated by 30 kGy and 5.5 and 4.5 log by 20 kGy. This equates to a D_{10} of 4.1–4.3 kGy. This study also reported that type C FMDV was reduced five logs by 30 kGy and 3.5 log by 20 kGy in liquid manure.

Smoke and Lombardo (2005) reported D_{37} values of 2.7 kGy for types A, O and C FMDV, equating to D_{10} of 6.2 kGy. Last et al. (1992) reported that doses of 15 kGy and 25 kGy were not sufficient to inactivate types A, O and C in processed meat product unless also heat treated.

Encephalomyocarditis virus (Genus Cardiovirus)

Miekka et al. (2003) reported a D_{37} of 1.4 for virus H161 in 25 per cent solution human albumin, which equates to a D_{10} of 3.2 kGy.

Poliovirus (Genus Enterovirus)

Sullivan et al. (1971) reported D_{10} values for polioviruses I-MASH, I-Lotshaw, II-Y-SK, II-Lansing, III-Leon and III-Nadler in Eagle's MEM with 2 per cent FBS of 4.9, 5.3, 4.1 to 5.4, 4.1 to 5.4, 4.1 to 5.4, and 4.8 kGy, respectively. Further trials involving over 100 observations confirmed a similar D_{10} range for III-Leon of 4.0 to 4.8 kGy. However, the D_{10} for virus (III-Leon) in water was significantly lower at 1.1 kGy.

Jordan and Kempe (1956) report a D_{10} of 7.57 kGy of poliovirus (Lansing strain) in brain and 6.2 kGy for crude virus. Pruss et al. (2001) reported a D_{10} of 7.1 kGy for poliovirus (PV-1) as frozen virus suspensions in plastic tubes. Pruss et al. (2001) also reported D_{10} values ranging from <4.4 to 5.2 kGy for poliovirus (PV-1) in bone (contaminated diaphyses).

A study into the effect of irradiation on coagulation proteins reported an inactivation rate of 0.204 $\log_{10}$ $TCID_{50}$ doses/mL/kGy for the Sabin vaccine strain of polio virus 1 which is estimated to be a D_{10} of 4.90 kGy (Kitchen et al. 1989).

Heidelbaugh et al. (1968) showed a D_{10} value of 6 kGy for poliovirus antibody (Ab) sabin inoculated into frozen filleted fish. The data showed that approximately tenfold greater drop in virus titre in the fish fillet compared to that of the poliovirus type one strain LS Ab sabin suspended in Eagle's basal medium with Hank's balanced salt base (showed a reduction of only 0.1×10^4 in titre).

Coxsackieviruses (Genus Enterovirus)

Sullivan et al. (1971) reported D_{10} values for coxsackieviruses A-9, A-11, B-1, B-2, B-3, B-4 and B-5 in Eagle's MEM with 2 per cent FBS of 4.2, 4.8, 4.1 and 4.4 kGy, respectively. Further trials involving more than 100 observations confirmed similar D_{10} values for A-9 and B-2 as 4.6 and 4.5 kGy, respectively. However, the D_{10} for viruses (A-9 and B-2) in water were significantly lower at 1.2 and 1.4 kGy.

Sullivan et al. (1973) attempted to demonstrate the effect of environment and temperatures on the effectiveness of gamma irradiation. The study reported D_{10} values, as determined by plaque assay for Coxsackievirus B-2 in Eagle's MEM containing 32 per cent FBS at –30, –60 and –90°C of 6.9 kGy, 5.9 kGy and 6.4 kGy respectively and a D_{10} of 5.3 kGy for the virus in water at –90°C. The same study reported D_{10} values for Coxsackievirus B-2 in cooked beef of 7.0 kGy, 7.6 kGy, 6.8 kGy, 7.8 kGy and 8.1 kGy at temperatures of 16, 0.5, –30, –60 and –90°C

respectively. It also reported D_{10} values in raw beef of 7.5 kGy, 7.1 kGy and 6.8 kGy at temperatures of –30°C, –60°C and –90°C, respectively.

Echoviruses (Genus Enterovirus)

Sullivan et al. (1971) reported D_{10} values for echoviruses 4, 5, 6, 7, 9, 11, 12 and 18 in Eagle's MEM with 2 per cent FBS of 4.6, 4.9, 5.1, 3.8 to 5.1, 5.0, 4.2 to 5.0, 5.0 and 4.4 kGy, respectively. However, the D_{10} for virus (echovirus 11) in water was significantly lower at 1.4 kGy.

Porcine enteroviruses 8–10 (Genus Enterovirus)

Preuss (1997) demonstrated a D_{10} of 6.4 kGy in frozen bovine serum and 4.4 kGy in liquid serum using e-beam radiation.

Swine vesicular disease (Genus Enterovirus)

Thomas et al. (1981) reported 5.5 kGy for one log reduction in cell culture medium. Thomas et al. (1982) reported D_{10} of 5.4 kGy for virus in chopped and frozen epithelial tissue from infected pigs, 4.8 kGy for virus in chopped and frozen lymph tissue from infected pigs, and 3.8 kGy for virus in cell culture medium and cellular debris, mixed wet with sewage sludge.

House et al. (1990) reported 5.0 kGy for one log reduction in bovine serum at –68°C using a TCID assay. According to Groneman et al. (1977), the D_{10} value for swine vesicular disease virus irradiated in cell culture at 0°C was 5.9 kGy and virus mixed in sewage sludge had a D_{10} of 6.2 kGy. Unlike the study's results for FMDV, sewage sludge did not appear to have a significant protective effect against irradiation on swine vesicular disease virus.

Simon et al. (1983) reported that 6 log of extracellular virus and cell associated swine vesicular disease virus was inactivated by 30 kGy, and 4 and 5.5 log by 20 kGy. This equates to a D_{10} of 4 to 5 kGy. This study also reported that swine vesicular disease virus was reduced 7 log by 30 kGy and 2 log by 20 kGy in liquid manure equating to a D_{10} of 3.85 kGy.

Hepatitis A (Genus Hepatovirus)

Pruss et al. (2001) reported a D_{10} of 5.3 kGy for frozen hepatitis virus A suspensions in plastic tubes and <4.6 to <4.7 for virus in bone (contaminated diaphyses).

Bidawid et al. (2000) reported D_{10} values of 2.72 and 2.97 kGy for hepatitis A virus in fruit and vegetables.

Teschen disease virus (Genus Teschovirus)

Thomas et al. (1981) reported that 2.8 kGy achieved a one log reduction of porcine enterovirus 1 (Teschen disease) in cell culture medium. Simon et al. (1983) also reported five log reduction by 10 kGy and a seven log reduction by 20 kGy and 30 kGy. This equates to a D_{10} ranging from 2 to 4.3 kGy.

Conclusion

Based on the available studies, the D_{10} range for viruses in the family Picornaviridae is estimated to be 1.2–8.1 kGy. However, due to extreme consequences of an FMD outbreak, careful consideration of product parameters (for example, product

radiodensity, homogenicity, temperature, water and oxygen level) and certification confidence is especially required prior to approval of irradiation and the dose to be used as a quarantine measure for product from FMD risk countries.

Family Polyomaviridae

Until recently, the family of Polyomaviridae contained only one genus (Polyomavirus). The family has been reclassified into 3 genera: Orthopolyomavirus, Wukipolyomavirus, and Avipolyomavirus.

Virus structure

Virions consist of a capsid. Virus capsid is not enveloped, round with icosahedral symmetry. The genome is not segmented and contains a single molecule of circular, supercoiled dsDNA that forms a covalently closed circle. The capsid is isometric and has a diameter of 40–55 nm.

Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.34 g cm^{-3}.

Viruses

Simian virus 40 and polyoma virus 12 (Genus Orthopolyomavirus)

Sullivan et al. (1971) reported 3.9 to 4.5 kGy for one log reduction of simian virus 40 in Eagle's MEM plus 2 per cent FBS. Basilico et al. (1965) demonstrated approximately four log reduction of polyomavirus 12 by 48 kGy.

Conclusion

Based on the available studies, the D_{10} range for viruses in the family Polyomaviridae is estimated to be 3.9–12.0 kGy.

Family Poxviridae

The Family Poxviridae contains two subfamilies: Chordopoxvirinae and Entomopoxvirinae. The Subfamily Chordopoxvirinae contains eight genera: Avipoxvirus, Capripoxvirus, Leporipoxvirus, Molluscipoxvirus, Orthopoxvirus, Parapoxvirus, Suipoxvirus and Yatapoxvirus.

The subfamily Entomopoxvirinae contains four genera: Alphaentomopoxvirus, Betaentomopoxvirus, Entomopoxvirinae and Gammaentomopoxvirus. Some viruses are unassigned under the subfamily Entomopoxvirinae and the family Poxviridae.

Virus structure

Virions consist of an envelope, a surface membrane, a core, and lateral bodies, or a surface membrane, a core, and lateral bodies. Virions are generally ovoid and brick-shaped, or pleomorphic measuring 140–260 nm in diameter, or 160–190 nm in diameter (ovoid, 220–450 nm in length, or 250–300 nm in length, 140–260 nm in height). The genome is not segmented and contains a single molecule of linear dsDNA.

Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.23–1.27 g cm^{-3}.

Viruses

Vaccinia (Genus Orthopoxvirus)

Lowy (2005) summarised the D_{10} values for various strains of vaccinia virus from various studies as follows:

IHD strain:	D_{10} = 4.18 kGy
Lancy strain:	D_{10} = 8.14 kGy
Levanditi strain:	D_{10} = 2.62 kGy
Merieux 37 (Lister) strain:	D_{10} = 6.04 kGy
Rabbit strain:	D_{10} = 5.25 kGy

Miekka et al. (2003) reported a D_{37} of 0.9 kGy of Copenhagen strain in 25 per cent solution human albumin equating to a D_{10} of 2.12 kGy. Jordan and Kempe (1956) reported a D_{10} of 4.47 kGy for Armstrong strain in brain and 3.88 kGy as crude virus.

Avian poxvirus (Genus Avianpox)

Thomas et al. (1981) reported a D_{10} of 2.2 kGy for avian pox virus. Richmond (1981) quoted a D_{10} of 1.87 kGy of avian pox virus in suspension.

Lumpy skin disease virus (Genus Capripoxvirus) and orf virus (Genus Parapoxvirus)

No information was found regarding lumpy skin disease virus and orf virus (contagious pustular dermatitis). However, the previously mentioned information regarding vaccinia virus and avian pox could be extrapolated to lumpy skin and orf viruses as these viruses are in the same Poxviridae family.

Conclusion

Based on the available studies, the D_{10} range for viruses in the family Poxviridae is estimated to range from 1.70 to 8.14 kGy. However, because of the very high consequences of lumpy skin disease and capripox, and the lack of data on the effectiveness of irradiation against these pathogens, careful consideration of product parameters (for example, product radiodensity, homogenicity, temperature, water and oxygen level) and certification confidence is especially required prior to approval of irradiation and the dose to be used as a quarantine measure for product from high risk countries.

Family Reoviridae

The family Reoviridae contains eleven genera: Aquareovirus, Coltivirus, Cypovirus, Fijivirus, Idnoreovirus, Orbivirus, Orthoreovirus, Oryzavirus, Phytoreovirus, Rotavirus and Seadornavirus.

Virus structure

Reovirus virions are non-enveloped and consist of a capsid, a core, and a nucleoprotein complex. The capsid is 60–80 nm in diameter and is icosahedral. The reovirus genome is monomeric and consists of 10 to 12 segments (depending on the genus) of linear dsRNA.

Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.26–1.44 g cm^{-3}.

Viruses

Reovirus (Genus Orthoreovirus)

Sullivan et al. (1971) reported 4.2 to 4.4 kGy for one log reduction of reovirus 1 in Eagle's MEM plus 2 per cent FBS. Willkommen et al. (1999) reported that reovirus in serum was reduced 6.7 log $TCID_{50}$ by 25 kGy equating to a D_{10} of 3.73. Richmond (1981) quoted a D_{10} for reovirus in suspension of 4 kGy.

Hermann (2005) reported a 6.7 log reduction of reovirus 3 was achieved by 35 kGy. This equates to a D_{10} of 5.2 kGy. The study also looked at effect of UV treatment (four log reduction by 254 nm UV-C) and combined treatment (10.7 log reduction).

Bluetongue virus (Genus Orbivirus)

Thomas et al. (1981) reported 2.0 kGy for one log reduction of bluetongue virus in whole bovine blood. Thomas et al. (1982) also reported a D_{10} of 2 kGy for the virus in whole sheep chilled blood from viraemic sheep with added ethylene-diamine-tetra-acetic acid (EDTA).

Willkommen et al. (1999) reported that bluetongue virus in FCS was reduced 3.8 log $TCID_{50}$ by 25 kGy. As there were only two data points, the estimated D_{10} of 6.58 kGy may not be reliable. House et al. (1990) reported 8.3 kGy for one log reduction in bovine serum at –68°C using a TCID assay.

Thomas and Samagh (1984) reported a D_{10} of less than 2 kGy for bluetongue virus in whole blood at 4°C; 1.1 kGy in cell culture at 4°C; 4.3 kGy in cell culture at –190°C; 2 kGy in mouse brain at 4°C; and 4.8 kGy in mouse brain at –190°C.

House et al. (1990) reported 8.3 kGy for one log reduction of bluetongue virus suspended in bovine serum at –68°C using a TCID assay.

Purtle et al. (2006b) reported a D_{10} value ranging from 7.5 to 10.5 kGy in foetal calf serum.

The reported D_{10} values for bluetongue virus ranged from 1 to 10.5 kGy. SAFC Biosciences (2006) reported inactivation of >3 logs at 25–35 kGy.

African horse sickness (Genus Orbivirus)

Thomas and Samagh (1984) reported a D_{10} of 1.7 kGy for African horse sickness virus in cell culture at 4°C; 3.4 kGy in cell culture at –190°C; 1.9 kGy in mouse brain at 4°C and 4.8 kGy in mouse brain at –190°C.

Conclusion

Based on the available studies, the D_{10} range for viruses in the Reoviridae family is estimated to range from 1.0 to 10.5 kGy.

Family Retroviridae

The family Retroviridae contains two subfamilies: Orthoretrovirinae and Spumaretrovirinae. The subfamily Orthoretrovirinae contains six genera:

Alpharetrovirus, Betaretrovirus, Deltaretrovirus, Epsilonretrovirus, Gammaretrovirus and Lentivirus. The subfamily Spumaretrovirinae contains one genus, Spumavirus.

Virus structure

Virions consist of an envelope, a nucleocapsid, and a nucleoid. Virus capsid is enveloped. Virions are spherical to pleomorphic. Virions measure 80–100 nm in diameter. The genome is positive-sense ssRNA.

Physicochemical and physical properties

Virions have a buoyant density in sucrose of 1.13–1.18 g cm^{-3}.

Viruses

Feline leukemia virus (Genus Gammaretrovirus)

Withrow (1990) reported incomplete inactivation of feline leukemia virus in infected bone by 29 kGy.

Human immunodeficiency virus (Genus Lentivirus)

Fideler et al. (1994) reported incomplete inactivation of human immunodeficiency virus (HIV) in cadaver-derived, infected bone grafts by 20/25 kGy but virus was undetectable after 30–40 kGy. Salai et al. (1997) reported that HIV type 1 infected T cells in bone and cell-free virus was inactivated at 25 kGy. Campbell and Li (1999) reported that 35 kGy is needed to inactivate the HIV bioburden in bone allografts and that 89 kGy is needed for a 6 log SAL. The inactivation rate of irradiated virus was 0.1134 $\log_{10}$ $TCID_{50}$/mL per kGy.

In a study on the effect of gamma irradiation on plasma and coagulation factors, 5–6 log of HIV was inactivated by 50–100 kGy at –80°C in frozen plasma and by 25 kGy at 15°C in liquid plasma (Hiemstra et al. 1991). Another study into the effect on HIV and coagulation proteins reported an inactivation rate of 0.164 $\log_{10}$ $TCID_{50}$ doses/ml/kGy (Kitchen et al. 1989). This is estimated to be a D_{10} of 6.1 kGy. HIV-2 in frozen virus suspensions in plastic tubes was reported to have a D_{10} of 7.1 kGy and up to 8.9 kGy for virus in contaminated bone diaphyses (Pruss et al. 2001).

Smith et al. (2001) reported that HIV type 1 in supernatant survived 50 kGy and Fideler et al. (1994) reported that HIV survives 25 kGy but is inactivated by at least 30 kGy in frozen bone, patella and ligament allografts from infected cadavers. Often studies using infected cadaver-derived tissues do not provide details of viral titre involved.

Maedi-visna virus (Genus Lentivirus)

Thomas et al. (1981) reported 3.5 kGy for one log reduction in cell culture medium.

Conclusion

Based on the available studies, the D_{10} range for viruses in the Retroviridae is estimated to range from 1.3 to 10.6 kGy.

Family Rhabdoviridae

The Family Rhabdoviridae contains six genera: Cytorhabdovirus, Ephemerovirus, Lyssavirus, Novirhabdovirus, Nucleorhabdovirus and Vesiculovirus.

Virus structure

Virions consist of an envelope and a nucleocapsid. Virus capsid is enveloped. Virions are in unfixed preparations bullet-shaped, or bacilliform (in cases of plant viruses when fixed prior to negative staining), or pleomorphic. Virions measure 45–100 nm in diameter and 100–430 nm in length (ICVTB 2006). The genome is not segmented and contains a single molecule of linear, usually NssRNA, or positive-sense full length ssRNA.

Physicochemical and physical properties

Virions have a buoyant density in sucrose of 1.14–1.2 g cm^{-3}.

Viruses

Vesicular stomatitis (Genus Vesiculovirus)

Thomas et al. (1981) reported that no vesicular stomatitis virus was recovered by egg inoculation with irradiated, contaminated liquid pig faeces although the results were undetermined whereas a D_{10} of 2 kGy was determined for virus in chopped, infected pig epithelial tissue.

Bovine ephemeral fever virus (Genus Ephemerovirus)

House et al. (1990) reported 2.9 kGy for one log reduction of bovine ephemeral fever virus in bovine serum at –68°C using a TCID assay.

Rabies (Genus Lyssavirus)

Gamble (1980) reported that 12.6 kGy eliminated infectivity from rabies strain CVS-11 adsorbing suspension, reducing infectivity titre in two-day-old mice from 5.5 $\log_{10}$/0.02 mL of intracranial inoculum to zero. A dose of 16.4 kGy also eliminated infectivity of rabies street virus antigen in brain suspensions, reducing infectivity from 6 $\log_{10}$/0.02 mL to zero. This equates to D_{10} values of 2.3 kGy for street strain and 2.7 kGy for the CVS-11 strain.

Conclusion

Based on the available studies, the D_{10} range for viruses in the family Rhabdoviridae is estimated to range from 2.0–2.9 kGy.

Family Togaviridae

The family Togaviridae has three genera: Alphavirus, Rubivirus and unassigned viruses.

Virus structure

Virions consist of an envelope and a nucleocapsid. During their life cycle, virions have not been observed outside a cellular environment and have a cell-associated cycle. Virus capsid is tightly enveloped by a detergent sensitive lipoprotein. Virions are spherical to pleomorphic. Virions measure 70 nm in diameter.

Physicochemical and physical properties

Virions have a buoyant density in sucrose of 1.18–1.2 g cm^{-3}. The sedimentation coefficient is 280 S20w. The thermal inactivation point is at 58°C. The longevity *in vitro* is 0.35 days (at 37°C in culture medium). Under *in vitro* conditions virions are

stable in an alkaline environment of pH 7–8. Virions are sensitive to treatment with organic solvents and detergents (which solubilise their lipoprotein envelope).

Viruses

Western equine encephalitis (Genus Alphavirus)

Jordan and Kempe (1956) reported a D_{10} of 5.17 kGy for Western equine encephalitis in brain material and 5.43 kGy for crude virus.

Venezuelan equine encephalitis (Genus Alphavirus)

Reitman and Tribble (1967) reported that D_{10} values for Venezuelan equine encephalitis varied significantly depending on titration method used. For example, minimal inhibitory concentration LD_{50}: 6.04 kGy; PFU: 7.15 kGy; and cytopathic effect 6.83 kGy. The report also noted that the D_{10} may be higher based on live suckling mice inoculation. Venezuelan equine encephalitis virus survived 60 kGy but not 80 kGy. Reitman et al. (1970) later reported that doses of 80 kGy and 100 kGy were required to inactivate 8.1 to 10 log of Venezuelan equine encephalitis virus (for vaccine use). However, there was insufficient data presented in this study to determine its D_{10} although it would be less than 10 kGy.

According to Smith (1990), the Trinidad strain of Venezuelan equine encephalitis was reduced seven log reduction by 32 x 10^5 rad (32 kGy), equating to a D_{10} of 4.57 kGy.

Sindbis (Genus Alphavirus)

Grieb et al. (2005) reported 4.9 log of Sindbis virus was reduced in pulverized bone by 50 kGy, equating to a D_{10} to 10.2 kGy.

Conclusion

Based on the available studies, the D_{10} range for viruses in the family Togaviridae is estimated to range from 3.87 to 10.2 kGy.

Recommendation – viruses

- Where irradiation treatment is considered necessary to address animal biosecurity concerns, an irradiation dose of 50 kGy should continue to be used unless otherwise determined as follows:
- The maximum D_{10} value as listed in Table 8, Appendix 3 for the relevant viral pathogen may be used in association with the recommended SAL to determine an appropriate gamma radiation dose to address specific animal biosecurity issues.
- In the absence of data for the specific virus species, the maximum D_{10} value for the family may be used (see Table 2).
- Where there is more than one viral pathogen of biosecurity concern, the maximum D_{10} value of the most radio-resistant virus should be used.
- Approval to use irradiation as an animal biosecurity option to address exotic disease concerns should take into consideration product parameters relevant to the effectiveness of irradiation (for example, radiodensity of materials, homogenecity of product) environmental factors (for example, water and

oxygen levels), and if necessary , the animal health status of the country of origin, other processing treatments and certification confidence.

Radiosensitivity of bacteria

There is considerable variation in the susceptibility to ionising radiation between species and even between strains of bacteria. Environmental factors such as media used, water activity and temperature during treatment and the bacteria's form (i.e. spore versus vegetative) also significantly affect radiosensitivity. Table 3 presents the radiation resistance of selected bacteria in fresh and frozen foods of animal origin.

Even within bacterial families, radio-resistance may vary considerably. For example, Reid and Fairand (1998) quoted a French study (Dupuy and Tremeau 1961) as reporting the D_{10} values derived from the survival curves of 21 strains of *Lactobacillus* ranged from 0.05 to 0.14 kGy. However, Comer et al. (1963) reported that 18 serotypes of *Salmonella* had a narrow D_{10} range of 0.52 to 0.77 kGy. In this study the dose levels required for a seven log reduction of the 18 serotypes ranged from 3.6 to 5.4 kGy. A review of the literature conducted by Nims *et al.* (2011) on *mycoplasma* (*orale, pneumoniae, and hyorhinis*) showed that the D_{10} values may range from 3.76-5.39 in frozen calf serum. Purtle *et al.* (2006b) reported D_{10} values ranging from 1.16 - 1.78 for *Acholeplasma laidlawi* in frozen foetal bovine serum.

According to Sztanyik (1974), vegetative gram-negative bacteria, having D_{10} values ranging between 0.029 to 0.24 kGy, are more radiosensitive than gram-positive bacteria with D_{10} ranging between 0.18 to 0.89 kGy. Bacterial spores are considered much more resistant to ionising radiation than vegetative bacteria. The cortex may osmotically remove water from the interior of the endospore and the dehydration that results is thought to be very important in the endospore's resistance to radiation. Also the small acid-soluble proteins within endospores saturate the endospore's DNA and protect it from radiation.

The D_{10} value for the anaerobic spore-former *Clostridium* spp. ranges between 2.2 and 5.4 kGy, and for the aerobic spore-former *Bacillus* spp. between 1.2 and 5.0 kGy.

Some bacteria possess exceptional DNA excision repair and DNA recombination ability thereby displaying unusual radiation resistance. An example is *Micrococcus radiodurans* which has a D_{10} value reportedly reaching 10 kGy.

The effect of the media during treatment is demonstrated by *Salmonella* Typhimurium with a D_{10} value of 0.21 kGy in phosphate buffer and 1.74 kGy in fish meat (Ley 1973 as quoted by Gazsó and Gyulai 2004). A Centers for Disease Control and Prevention report (Tauxe 2001) quoted the D_{10} value of *Salmonella* at refrigerator temperature to be 0.70 kGy. This report also quoted D_{10} values for Campylobacter (0.20 kGy), *Cl. botulinum* spores (3.60 kGy), *Escherichia coli* O157 (0.30 kGy) and *Listeria* (0.45 kGy).

Garin-Bustagi *et al.* (1990) showed that in frozen bovine colestrum, *Brucella abortus* has a D_{10} value of 0.035 kGy, *E. coli* K99 and *Salmonella dublin* 0.02 kGy and *Mycobacterium paratuberculosis* 0.06 kGy.

The potential of certain pathogens to be used for bioterrorism was highlighted by the anthrax incidents in the USA in 2001 which also generated interest in the use of irradiation to sanitise mail. Carter and Verrelli (1973; cited in Elliott et al. 2005) reported D_{10} values for the following *Bacillus anthracis* simulants using gamma irradiation:

Bacillus anthracis Sterne	1.1 kGy (dry); 2.7 to 4.3 kGy (wet)
Bacillus atrophaeus	1.1 to 2.0 kGy (dry); 2.1 kGy (wet)
Bacillus pumilus	1.4 kGy (dry); 2.8 kGy (wet)
Bacillus thuringiensis	1.2 kGy (dry); 2.0 kGy (wet)

Carter and Verrelli (1973; cited in Elliott et al. 2005) also demonstrated that a small proportion of *Bacillus anthracis* and *Bacillus atrophaeus* populations were radio-resistant, both with a D_{10} of 5.7 kGy.

Bowen et al. (1996) reported a D_{10} of 5.5 kGy for *Bacillus anthracis*. Niebuhr and Dickson (2003) demonstrated a D_{10} value of 3.35 kGy for *Bacillus anthracis* strain Sterne 34F2 spores contained in non-fat dry milk powder using electron beam radiation.

Horne et al. (1959) recommended that a minimum dose of 20 kGy would provide a sufficient margin of safety in an industrial process for inactivation of *B. anthracis* spores in baled goat hair. OIE Code (2011) specifies a irradiation dose of 40 kGy for disinfection of *B. anthracis* spores in skins and trophies in wild animals and 25 kGy for wool and hair.

Table 3. Gamma irradiation resistance of selected bacteria in foods (Farkas 2007)

Microorganism	**D_{10} value (kGy)**	
Vegetative cells	**Fresh food**	**Frozen food**
Aeromonas hydrophila	0.14–0.19	
Bacillus cereus	0.17	
Brucella abortus	0.34	
Campylobacter jejuni	0.08–0.20	0.21–0.32
Clostridium perfringens	0.59–0.83	
Escherichia coli (incl. O157:H7)	0.23–0.35	0.3–0.6
Lactobacillus spp.	0.3–0.9	
Listeria monocytogenes	0.27–1.0	0.52–1.3
Moraxella phenylpyruvica	0.63–0.88	
Proteus vulgaris	0.20	

Microorganism	D_{10} value (kGy)	
Vegetative cells	**Fresh food**	**Frozen food**
Pseudomonas putida	0.06–0.11	
Salmonella spp.	0.3–0.8	0.4–1.3
Shigella spp.		0.2–0.4
Streptococcus faecalis	0.65–1.0	
Staphylococcus aureus	0.26–0.6	0.3–0.45
Vibrio spp.	0.03–0.12	0.11–0.75
Yersinia enterocolitica	0.04–0.21	0.4
Clostridium botulinum type E (spores)	1.25–1.40	
Bacillus cereus	1.6	
Clostridium sporogenes	1.5–2.2	
Deinobacter spp.	5.05	
Deinococcus radiodurans	3.1–5	

A comprehensive list of D_{10} values for bacteria is presented in Table 9, Appendix 3.

Recommendation – bacteria

- There are few exotic bacterial species of animal biosecurity concern for Australia. For most biosecurity scenarios, it is necessary to address viral pathogens of concern that typically have a significantly higher D_{10} value than bacteria—most bacteria seem to require D_{10} less than 5 kGy. However, where it is only necessary to address bacteria, the maximum value listed in Table 9, Appendix 3, for the specific bacteria of concern could be used in association with the recommended SAL to determine an appropriate gamma irradiation dose to address specific animal biosecurity issues with the bacteria.

- If the specific bacterial species is not listed, the maximum D_{10} for the bacterial genus should be used.

Radiosensitivity of fungi

The radiation sensitivity of many fungi is of the same order of magnitude as that of vegetative bacteria and is considerably less than that of bacterial spores. Yeasts are as resistant as the more resistant bacteria (Farkas 2006) whereas fungi with melanised hyphae have a radiation resistance comparable to that of bacterial spores (Saleh et al. 1988). Although relatively high doses may be required to kill fungi, lower doses may be sufficient to prevent reproduction. Table 4 lists the gamma irradiation resistance of selected species of fungi.

Most studies of the inactivation of fungi by irradiation have been made on asexual spores. Germinating spores, mycelia and other morphological structures of fungi might have different radiation responses (Sommer 1973). The radiation sensitivity of fungi is influenced not only by genetic factors but also by the number of cells in a spore (effect of multicellularity) and the number of nuclei per cell (effect of multinuclearity). The haploid yeast cells are more sensitive than diploid ones (effect of ploidity).

Ten species of fungi representing the genera Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium and Penicillium were examined by Saleh et al. (1988). The D_{10} value of fungal conidia in water for *Aspergillus niger* was 0.420 kGy, for *Cladosporium cladosporoides* 0.300 kGy and for *Curvularia geniculata* 0.290 kGy. D_{10} values for dematiaceous fungi (in agar medium) ranged from 6 to 17 kGy and for moniliaceous fungi were less than 3 kGy.

Table 4. Gamma irradiation resistance of selected fungal species

Species	Media	D_{10} value (kGy)	Reference
Aspergillus echinulatus	Water	0.319	Blank and Corrigan (1995)
Aspergillus flavus	Water	0.55–0.60	Saleh (1988)
Aspergillus fumigates	Margarine	1.08	Gumus et al. (2008)
Aspergillus fumigates	Water	0.276	Blank and Corrigan (1995)
Aspergillus glaucus	Water	0.25	Blank and Corrigan (1995)
Aspergillus niger	Saline + 5% gelatin	0.5	Yusof (2007)
Aspergillus niger	Water	0.245	Blank and Corrigan (1995)
Aspergillus niger	Water	0.42	Saleh et al. (1988)
Aspergillus ochraceus	Water	0.209	Blank and Corrigan (1995)
Aspergillus versicolour	Water	0.282	Blank and Corrigan (1995)
Candida zeylandoides	Chicken skin	0.68	Hughes (1991) as quoted by Patterson (2005)
Cladosporium cladosporioides	Water	1.798	Blank and Corrigan (1995)
Cladosporium cladosporioides	Water	0.25–0.30	Saleh et al.(1988)
Curvularia geniculata	Water	2.42–2.90	Saleh et al.(1988)
Paecilomyces variotii	Margarine	0.59	Gumus et al.(2008)
Penicillium aurantiogriseum	Water	0.236	Blank and Corrigan (1995)

Species	Media	D_{10} value (kGy)	Reference
Penicillium cyclopium	Water	0.397	Blank and Corrigan (1995)
Penicillium expansum	Grain	0.32	O'Neill et al. (1991) as quoted by Patterson (2005)
Penicillium granulactum	Water	0.239	Blank and Corrigan (1995)
Penicillium notatum	Saline + 5% gelatin	0.2	Yusof (2007)
Penicillium roqueforti	Water	0.416	Blank and Corrigan (1995)
Penicillium verrocusum	Water	0.266	Blank and Corrigan (1995)
Penicillium viridicatum	Water	0.333	Blank and Corrigan (1995)
Saccharomyces cerevisiae	Saline + 5% gelatin	0.5	Yusof (2007)
Torulopis candida	Saline + 5% gelatin	0.4	Yusof (2007)

Although a substantial amount of data is available in the literature, most have been obtained under different experimental conditions. Due to the considerable effect of environmental condition on the actual radiosensitivity, achieving a correct comparison is very difficult.

Recommendation – fungi

- There are few, if any, exotic fungal species of animal biosecurity concern for Australia.
- For most biosecurity scenarios, it is necessary to address viral pathogens of concern which typically have a significantly higher D_{10} value than fungi. However, should a fungal species be identified as a biosecurity concern and in the absence of specific data for the organism or its genus, a D_{10} value of 2.90 kGy could be used in association with the recommended SAL to determine an appropriate gamma irradiation dose to address the biosecurity concern.

Radiosensitivity of TSE agents

TSE infective agents (prions) are infectious proteins which cause a group of fatal neurodegenerative diseases. These prion diseases present as either genetic, infectious or sporadic disorders, all involving modification of the prion protein (PrP). Examples of TSEs in animals include bovine spongiform encephalopathy, chronic wasting disease, scrapie and transmissible mink encephalopathy. In humans, TSEs

include Creutzfeldt-Jakob disease (CJD), fatal familial insomnia, kuru and Gerstmann-Sträussler-Scheinker syndrome.

Prions are devoid of any nuclear material and are comprised entirely of a modified protein (PrP^{sc}). The normal cellular protein (PrP^{c}), which is characterised by a structure of four helixes (α-helixes), is converted to PrP^{sc} during which two of the helixes are converted to linear structures (β-sheets). It appears that PrP^{sc} acts as a template upon which the normal PrP^{c} form is refolded into PrP^{sc}.

Prions are resistant to irradiation as they are very small proteins, approximately 30 kDa in size and devoid of nucleic acid that could be degraded by ionising radiation. Gamma irradiation at 25 kGy could not eliminate prions from contaminated lyophilised dura mater (Hilmy and Pandansari 2007). Ionising radiation has little effect on TSE agents and has no practical application in their inactivation (Taylor 2000). Table 5 shows the gamma irradiation resistance of the TSE agents.

Table 5. Gamma irradiation resistance of TSE agents

TSE agent	Dose (kGy)	Estimated $\log_{10}$ titre reduction*	Reference
CJD (A-200 strain)	150	No reduction	Gibbs, Jr. et al. (1978)
CJD (O-39 strain)	150	No reduction	Gibbs, Jr. et al. (1978)
CJD (S-27 strain)(Gibbs, Jr. et al. 1978)	150	No reduction	Gibbs, Jr. et al. (1978)
CJD (cat-5 strain)	200	<1	Gibbs, Jr. et al. (1978)
Kuru (Eiru strain)	200	No reduction	Gibbs, Jr. et al. (1978)
Kuru (Sepe strain)	200	2	Gibbs, Jr. et al. (1978)
Kuru (Kigea strain)	200	3	Gibbs, Jr. et al. (1978)
Scrapie (C506 strain - primate adapted)	100	<2	Gibbs, Jr. et al. (1978)
Scrapie (C506 strain - mouse adapted)	100	<1.7	Gibbs, Jr. et al. (1978)
Scrapie (SPG9:M9 strain - mouse adapted)	50	No reduction	Gibbs, Jr. et al. (1978)
Scrapie (SPG9:M10 strain - mouse adapted)	150	<0.4	Gibbs, Jr. et al. (1978)
Scrapie (hamster adapted)	50	1.5	Miekka et al.(2003)

*Due to the long incubation periods of TSEs, the actual titre reductions may have beeen substantially less if the trial period had been extended.

Recommendation – TSE agents

- Ionising radiation is not recommended as a practical treatment to address biosecurity issues with TSE agents.

Radiosensitivity of foodborne parasites

Radiation effects on specific foodborne parasites have been investigated (Loaharanu and Murrell 1994). However, the available literature does not provide D_{10} values or reduction in titres for the parasitic agents. A summary of available information for irradiation of parasites is presented in Table 6. Only doses <10 kGy are needed for inactivation of fish-borne, snail-borne and crustacean-borne parasites, *Angistrongylus* species, *Anisakis* species, *Ascaris lumbricoides* eggs, *Entamoeba histolytica, Heterophyes* spp., *Hymenolepis nana,* liver flukes, *Paragonimus* spp., and *Trichina* spp. WHO (1999) suggests that sequential heating and freezing plus high irradiation doses will inactivate parasites in food.

Irradiation of protozoa

Irradiation at an appropriate level will inhibit the division of living cells including protozoa. The sensitivity of protozoa to radiation varies according to species, stage of development and irradiation conditions. Table 6 provides details of irradiation doses required for various protozoans.

Irradiation of trematodes

Studies conducted under the program on the use of irradiation to control infectivity of foodborne parasites (International Atomic Energy Agency et al. 1993) showed that minimum effective doses (MEDs) required to eliminate the infectivity of trematodes are well below 1.0 kGy (see Table 6). Studies conducted include irradiation of *Clonorchis sinensis, Opisthorchis vioverrini* in fish and *Paragonium westermani* in crabs.

Irradiation of cestodes

Available literature shows that cestodes require a MED of 0.2– >3.0 kGy.

Irradiation of nematodes

Of all the nematodes, *Anisakis* spp. seems to be highly resistant requiring a MED of 10 kGy for inactivation. The range for nematodes varies from 0.15–10 kGy for available parasites.

Conclusions

Generally, parasites seem to require lower levels of irradiation than viruses. WHO recommends high dose irradiation in combination with heat treatment or freezing for inactivation of parasites in food. As available information provides MED rather than D_{10} values or reduction in titres for parasites, a D_{10} value cannot be recommended for each parasite. Although almost all foodborne parasites are inactivated using <10 kGy MED, it should be noted that *Cryptosporidium parvum* is exceptionally resistant and requires a dose of 50 kGy MED to be inactivated.

Recommendation – foodborne parasites

- For most biosecurity scenarios, it is necessary to address viral pathogens of concern which generally requires higher doses than for parasites—most parasites and their various stages seem to require MEDs <10 kGy for inactivation based on inactivation data for foodborne parasites (see Table 6). Thus, irradiation doses required to manage animal biosecurity issues

associated with viruses in products will also address biosecurity issues associated with parasites and other stages of their life cycles.

- There are few exotic parasitic species of animal biosecurity concern for Australia. However, should a parasite be identified as a biosecurity concern, a dose of 25 kGy is recommended.

Table 6. Irradiation of parasites (protozoa, trematodes, cestodes, nematodes)

Parasites	MED (kGy)	Occurrence / mode of infection	Parasite stage	Substrate	Effect of irradiation	Bioassay model	Reference
Parasites carried by food							
Protozoa							
Toxoplasma gondii	0.3	Consumption of undercooked meat or poultry	Tachyzoites	Not available	Parasite killed	Mice	Baldelli et al. (1971) as quoted by Loaharanu and Murrell (1994)
	0.09	Consumption of undercooked meat or poultry	Parasite	Not available	Elimination of infectivity	Mice	Baldelli et al. (1971) as quoted by Loaharanu and Murrell (1994)
	0.5	Consumption of undercooked meat or poultry	Unsporulated oocysts	From muscle, tongue, heart and limbs of pigs	Elimination of infectivity	Mice, cats	Dubay et al. (1986)
	0.4	Consumption of undercooked meat or poultry	Unsporulated oocysts	Brains of mice	Elimination of infectivity	Mice, cats	Dubay and Thayer (1994)
	0.5	Consumption of undercooked meat or poultry	Oocysts	Fruits and vegetables	Elimination of infectivity	Mice	Dubay et al.(1998)

Parasites	MED (kGy)	Occurrence / mode of infection	Parasite stage	Substrate	Effect of irradiation	Bioassay model	Reference
Toxoplasma gondii	0.7	Consumption of undercooked meat or poultry	Not available	Meat	Elimination of infectivity	Mice, pigs	Wikerhouser (1991) as quoted by Loaharanu and Murrell (1994) Geerts et al. (1994)
	0.45–0.6	Consumption of undercooked meat or poultry	Oocysts	In experimentally infected mice and pig tissue	Elimination of infectivity	Mice, cats	Song et al. (1993)
	0.7	Consumption of undercooked meat or poultry	Not available	Not available	MED for fresh pork	Not available	Wilkinson and Gould (1996) as quoted by WHO (1999)
	0.3–0.7	Consumption of undercooked meat or poultry	Not available	Lamb and pork	Elimination of infectivity	Not available	Patterson (2005)
Trematodes							
Clonorchis sinensis	0.2	Chinese liver fluke, occurs in raw fish	Not available	Not available	Inhibits maturation	Guinea pigs	Chen (1991) as quoted by Loaharanu and Murrell (1994)

Parasites	MED (kGy)	Occurrence / mode of infection	Parasite stage	Substrate	Effect of irradiation	Bioassay model	Reference
Chlonorchis spp.	0.15	Chinese liver fluke, occurs in raw fish	Not available	Not available	*In vitro* MED	Not available	Wilkinson and Gould (1996) as quoted by WHO (1999)
Opisthorchis viverrini	0.15	Liver fluke found in contaminated raw pickled or smoked fish	Metacercaeriae	*In vitro*	Inhibits maturation	Guinea pigs, rats	Song et al. (1992) as quoted by Loaharanu and Murrell (1994)
	0.1	Liver fluke found in contaminated raw pickled or smoked fish	Not available	Not available	MED	*In vitro*	Wilkinson and Gould (1996) as quoted by WHO (1999)
	0.12	Liver fluke found in contaminated raw pickled or smoked fish	Metacercaeriae	*In vitro*	Inhibits maturation	Rabbits	Fu Shi Quan (2005)
Paragonimus westermani	0.1	Fluke in crabs	Metacercaeriae	Not available	Inhibits maturation	Hamsters	Sornmani et al. (1991) as quoted by Loaharanu and Murrell (1994)

Parasites	MED (kGy)	Occurrence / mode of infection	Parasite stage	Substrate	Effect of irradiation	Bioassay model	Reference
Paragonimus westermani	0.1	Fluke in crabs	Metacercaeriae	*In vitro*	Inhibits maturation	Adult cats and albino mice	Song et al. (1992)
Cestodes							
Taenia saginata	>3.0	Tapeworm found in uncooked or undercooked beef causes taeniasis	*Cysticercus bovis*	Meat	Complete inactivation of larvae	*In vitro* excystment	Van Kooy and Robjins (1968)
	0.3	Tapeworm found in uncooked or undercooked beef causes taeniasis	*Cysticercus bovis*	Not available	Preliminary MED	Not available	Wilkinson and Gould (1996) as quoted by WHO (1999)
	0.4	Tapeworm found in uncooked or undercooked beef causes taeniasis	Not available	Not available	Prevents development in humans	Human volunteers	Tolgay (1972) as quoted by Loaharanu and Murrell (1994)
	0.3	Tapeworm found in uncooked or undercooked beef causes taeniasis	Cysticerci	In minced meat	Elimination of infectivity	Human volunteers	Geerts et al.(1994), Alabey (1991) Verster (1979) as quoted by Loaharanu and Murrell (1994)

Parasites	MED (kGy)	Occurrence / mode of infection	Parasite stage	Substrate	Effect of irradiation	Bioassay model	Reference
Taenia solium	0.2–0.6	Tapeworm in pork	Cysticerci	*In vitro*	Elimination of infectivity	Hamsters	Geerts et al. (1994), Alabey (1991), Verster (1976) Verster (1979), as quoted by Loaharanu and Murrell (1994) and Singh and Dhar (1988)
	0.3	Tapeworm in pork	Cysticerci	Not available	Preliminary MED	Not available	Wilkinson and Gould (1996) as quoted by WHO (1999)
Hydatigera taeniaeformis	0.4–1.0	Not available	Eggs		Reduced infectivity	Mice	Williams and Colli (1972)
	0.7	Not available	Metacestode	Not available	Elimination of infectivity	Hamsters	de Aluja et al. (1994)
	0.65	Not available	Metacestode	*In vitro*	Elimination of infectivity	In vitro	de Aluja et al.(1994)
Echinococcus granulosus	2.0	Not available	Cysticerci	Meat	Elimination of infectivity	Unknown	Georgieva (1988)

Parasites	MED (kGy)	Occurrence / mode of infection	Parasite stage	Substrate	Effect of irradiation	Bioassay model	Reference
Echinococcus granulosus	0.5	Not available	Cysticerci	Unknown	Elimination of infectivity	Hamsters	Thakur (1991) as quoted by Loaharanu and Murrell (1994)
	0.4–06	Not available	Protoscolex		Elimination of infectivity	Dogs	Singh and Dhar (1988)
Taenia pisiformis	3.7– 4.6	Fruits and vegetables contaminated with cat faeces	Cysticerci	Sterile isotonic solution	MED	*In vitro*	Pawel (1968)
Nematodes							
Trichinella spiralis	0.3	Nematode occurs in raw or inadequately cooked pork	Larvae	Not available	Elimination of infectivity	Mice	Gould et al.(1953) Gomberg (1958) as quoted by Kraybill (1959)
	0.18	Nematode occurs in raw or inadequately cooked pork	Larvae	Not available	Stop development to adults	Not available	Gomberg (1958) as quoted by Kraybill (1959)
	0.3	Nematode occurs in raw or inadequately cooked pork	Not available	Not available	Elimination of infectivity	Rats	Gibbs (1964) as quoted by ICMSF (1996)

Parasites	MED (kGy)	Occurrence / mode of infection	Parasite stage	Substrate	Effect of irradiation	Bioassay model	Reference
Trichinella spiralis	0.3	Nematode occurs in raw or inadequately cooked pork	Larvae	Meat	Elimination of infectivity	Not available	Brake et al. (1985)
	0.11	Nematode occurs in raw or inadequately cooked pork	Not available	Not available	Sterilisation of females	Rats	Kraybill (1959)
	0.1–0.6	Nematode occurs in raw or inadequately cooked pork	Not available	Not available	Elimination of infectivity	Mice	Kasperzak (1959) as quoted by Loaharanu and Murrell (1994)
	0.15–0.7	Nematode occurs in raw or inadequately cooked pork	Not available	Pork	Elimination of infectivity	Not available	Patterson (2005)
Angiostrongylus cantonensis	2.0	Parasitic worm found in uncooked molluscs	Not available	Not available	Decreased infectivity	Mice	Oai (1991) and Chai (1991) as quoted by Loaharanu and Murrell (1994)

Parasites	MED (kGy)	Occurrence / mode of infection	Parasite stage	Substrate	Effect of irradiation	Bioassay model	Reference
Angiostrongylus cantonensis	4.0	Parasitic worm found in uncooked molluscs	Not available	Not available	Decreased infectivity	Mice	Oai (1991), Chai (1991) as quoted by Loaharanu and Murrell (1994)
	2.0	Parasitic worm found in uncooked molluscs	Not available	Not available	MED		Wilkinson and Gould (1996) as quoted by WHO (1999)
Gnathastoma spinigirum	7.0	Parasitic worms found in raw, undercooked or fermented fish	Not available	Not available	Reduced larval penetration	Rats	Setusban, pers. comm. as quoted by Loaharanu and Murrell (1994) Wilkinson and Gould (1996) as quoted by WHO (1999)
Anisakis sp.	6.0	Nematode ingested if fish is eaten raw or lightly salted	Not available	Not available	Reduced larval penetration	*In vitro* agar plate	Rutenburg (1971) as quoted by Loaharanu and Murrell (1994)

Parasites	MED (kGy)	Occurrence / mode of infection	Parasite stage	Substrate	Effect of irradiation	Bioassay model	Reference
Anisakis sp.	2–10	Nematode ingested if fish is eaten raw or lightly salted	Not available	Not available	Reduces infectivity of larvae	Unknown	Wilkinson and Gould (1996) as quoted by WHO (1999)
Paragonimus spp.	0.1	Parasitic worm found in crabs and crayfish in Asia	Not available	Not available	Reduces infectivity of larvae	*In vitro* MED	Wilkinson and Gould (1996) as quoted by WHO (1999)
Other parasites							
Protozoa							
Entamoeba histolytica	0.15	Not available	Cysts	Saline suspension	Effetiveness irradiation calculated using maximum probable number	Not available	Schneider (1960)
Cryptosporidium parvum	50	Not available	Oocysts	In distilled water		In mice	Yu and Park (2003)
Cestodes							
Hymenolepis nana	0.4–2.0	Cestode in mice	Eggs	Saline	Reduce infectivity of eggs	Mice Beetle	Onyango-Abuje and Weinmann (1974)

5 General principles of irradiation treatment as a biosecurity measure

Irradiation treatment is one of a number of risk management measures that the department considers when assessing animal biosecurity risk. An assessment of animal biosecurity risk is based on:

- the country and species of origin of each ingredient
- the processing of each ingredient and/or final product
- the end use of the product, and
- any other information relevant to the biosecurity risk.

Where a product presents an unacceptable biosecurity risk or does not meet import requirements, or where there is insufficient information to complete the assessment, importers may be given the option to irradiate. Irradiation is not intended to be a replacement to a production process, treatment or import policy but as an adjunct to address biosecurity concerns.

In cases where an importer can provide sufficient evidence to demonstrate that only certain pathogens of biosecurity concern could possibly be present in the product, the department may be able to determine more accurately the required irradiation dose. Determination of the dose would be based on this review and any other relevant data published in peer-reviewed literature.

Where irradiation treatment is necessary the following options may apply:

(1) use of an irradiation dose of 50 kGy

Where the risk assessment considers irradiation is necessary, a dose of 50 kGy should continue to be used as a routine animal biosecurity treatment unless otherwise determined by the biosecurity assessment of the import application.

(2) use of an irradiation dose lower than 50 kGy

If the biosecurity risk assessment of the import application determines that there are only a few animal pathogens of concern, the assessment may determine an irradiation dose lower then 50 kGy based on the D_{10} value of those pathogens and the SAL.

(3) use of an irradiation dose higher than 50 kGy

The biosecurity risk assessment may occasionally determine that, based on D_{10} values and the SAL, a dose higher than 50 kGy is required for products assessed as likely to be significantly contaminated with pathogens of animal biosecurity concern.

Due to the extreme consequences of some diseases (for example, FMD) and prior to granting approval and determining the irradiation dose to use on the product from

high risk countries, careful consideration should be given to relevant risk factors. These include product parameters (for example, radiodensity, homogeneity, temperature, water and oxygen levels), confidence in the information and certification, and potential for exposure of the product to susceptible species.

Chapter 3 of this review details how bio-burden is used with the SAL and D_{10} values to determine a recommended irradiation dose. Although it is difficult to estimate the bio-burden of contaminating microorganisms in the biological ingredients, tissues used in the manufacture of biological products would in most cases be derived from healthy animals which have passed ante and post-mortem inspection. For these products, the department assumes in most circumstances that the bio-burden would be close to zero. An example of where the bio-burden would be considered significantly higher than zero would be infected tissues imported into a laboratory for further research. In these situations, the department would consider the bio-burden in the infected tissue on a case-by-case basis and could recommend that product be irradiated with a higher dose.

APPENDIX 1 – Terminology

Sources: orise.orau.gov/reacts/guide/definitions.htm (accessed 29 March 2011)
hps.org/publicinformation/radterms (accessed 29 March 2011)

Atom: The smallest piece of an element that cannot be divided or broken up by chemical means.

Beta particle: A small particle ejected from a radioactive atom. It has a moderate penetrating power and a range of up to a few meters in air. Beta particles will penetrate only a fraction of an inch of skin tissue.

Curie (Ci): A unit of measure used to describe the amount of radioactivity in a sample of material. The unit of radioactivity is defined as 3.7×10^{10} decays per second.

***D_{37}* value**: The radiation dose at which approximately 37 per cent (36.79 per cent) of the initial entities are undamaged. It indicates the radiation required to inactivate 63 per cent of a population. It is the fluence producing on average one lethal hit per organism and reducing viable population to 37 per cent.

Dose: A general term for the quantity of radiation or energy absorbed.

Dose rate: The dose delivered per unit of time. It is usually expressed as rads per hour or in multiples or submultiples of this unit such as millirads per hour. The dose rate is commonly used to indicate the level of hazard from a radioactive source.

Dosimeter: A small, pocket-sized device used for monitoring radiation exposure of personnel. Before use, it is given a charge, and the amount of discharge that occurs is a measure of the accumulated radiation exposure.

Electromagnetic radiation: A traveling wave motion that results from changing electric and magnetic fields. Types of electromagnetic radiation range from those of short wavelength, like x-rays and gamma rays, through the ultraviolet, visible, and infrared regions, to radar and radio waves of relatively long wavelengths.

Exposure: A quantity used to indicate the amount of ionisation in air produced by x- or gamma ray radiation. The unit is the roentgen (R). For practical purposes, one roentgen is comparable to 1 rad or 1 rem for x and gamma radiation. The SI unit of exposure is the coulomb per kilogram (C/kg). One $R = 2.58 \times 10^{-4}$ C/kg.

Fluence: The number of particles (particle fluence) or quantity of energy (energy fluence) incident on a surface from all directions divided by the area of that surface.

Free radical: An atom or group of atoms (molecule) with an unpaired electron. Because they have a 'free' electron, free radicals are very unstable and highly reactive. The hydroxyl radical (OH) is an example of a free radical found in cells. In the body it is usually an oxygen molecule that has lost an electron and will stabilize itself by acquiring an electron from a nearby molecule.

Gamma rays or gamma radiation: Electromagnetic radiation of high energy. Gamma rays are the most penetrating type of radiation and represent the major external hazard.

Gray: The SI unit of radiation dose expressed in terms of absorbed energy per unit mass of tissue. The gray is the unit of absorbed dose and has replaced the rad. 1 gray = 1 Joule/kilogram and also equals 100 rad.

Ionisation: Production of charged particles in a medium. An orbital electron is stripped from a neutral atom, producing an ion pair (a negatively charged electron and a positively charged atom).

Ionising radiation: Electromagnetic (x-ray and gamma) or particulate (alpha, beta) radiation capable of producing ions or charged particles. Typically refers to heavy charged particles, fast electrons, neutrons, x-rays, and gamma rays.

Irradiation: Exposure to ionising radiation.

Isotope: Elements having the same atomic number (Z) and different mass number (A).

LD_{50}: the irradiation dose at which there is a 50 per cent chance of lethality.

Photon: a particle of zero rest mass moving with the speed of light and carrying electromagnetic momentum and energy. The photon energy (E) of monochromatic (single wavelength) electromagnetic radiation is related to the wavelength (l) by E = hc/l where h is Planck's constant (6.6262 $x10^{-34}$ J^{-s}) and c is the speed of light in a vacuum (2.9979 x 108 m/s).

Polymer: A naturally occurring or synthetic compound consisting of large molecules (macromolecule) made up of a linked series of repeating structural units typically connected by covalent chemical bonds. Although polymer in popular usage suggests plastic, the term actually refers to a large class of natural and synthetic materials with a variety of properties.

Rad: The original unit developed for expressing absorbed dose, which is the amount of energy from any type of ionising radiation (for example, alpha, beta, gamma, neutrons) deposited in any medium (for example, air, tissue, water). A dose of one rad is equivalent to the absorption of 100 ergs (a small but measurable amount of energy) per gram of absorbing tissue. The rad has been replaced by the gray as the SI units (1 gray = 100 rad). One rad of any ionising radiation corresponds to an energy absorption of 0.01 J/kg or 6.242 x 1013 eV/g.

Radiation: Energy traveling through space. Some types of radiation associated with radioactivity are alpha and beta particles and gamma and x-rays.

Radioactivity: The spontaneous emission of radiation from the nucleus of an unstable atom. As a result of this emission, the radioactive atom is converted, or decays, into an atom of a different element that might or might not be radioactive.

Radiation sensitivity (S): The radiation dose which induces a specified change in a biological system; see D_{37}.

Rep (Roentgen-equivalent-physical): A unit of absorbed radiation dose, equal to the amount of ionizing radiation that will transfer 93 ergs of energy to 1 gram of water or living tissue.

Roentgen (R): The unit of exposure from x or gamma rays. Exposure of dry air at 0^{o}C and 760 mm Hg to one roentgen of photon radiation produces ions of one sign carrying a total electrical charge of 2.58×10^{-4} C/kg of air when all secondary electrons are collected.

Sterility assurance level (SAL): The probability that a process makes something sterile. An SAL of 10^{-6} is the recommended probability of survival for organisms on a sterilised device or product. This level means that there is less than or equal to one chance in a million that an item remains contaminated or non-sterile.

Sievert (Sv): 1 sievert equals 100 rem.

Sub-lethal damage: Radiation damage which does not induce an endpoint scored as lethality.

Survival curve: A graph of the logarithm of the fraction of undamaged entities versus the dose.

Target theory: A phenomenological theory of radiation damage in which the number of 'hits' on a biological target is related to the radiation dose.

APPENDIX 2 – Conversion formula

Other measurement terms: SI units which may be used in place of the *rem* and the *rad* are the sievert (Sv) and the gray (Gy). These units are related as follows:
1Sv = 100 rem
1Gy = 100 rad
Two other terms which refer to the rate of radioactive decay of a radioactive material are curie (Ci) and becquerel (Bq).

Roentgen equivalent physicals (rep) = 93 erg/gram; 1 rep = 0.93 rad.

Gamma exposure:

1 roentgen (R) = 2.58 x 10^{-4} C/kg of dry air, where C is 1 coulomb

1 R = 1 esu/cm^3 of dry air, where 1 esu = 1 statC = 3.3356 × 10^{-10} C)

Absorbed dose:

1 radiation absorbed dose (rad) = 100 erg/g = 0.01 J/kg, where J = joule

1 gray (Gy) = 100 rad

1 Mrad = 10kGy

Equivalent dose:

1 roentgen equivalent man (rem) = 1 rad x Q (quality factor)

1 sievert (Sv) = 100 rem = 1 gray x Q

(for gamma and beta irradiation Q = 1)

Radioactivity:

1 curie (Ci) = 3.7 x 10^{10} becquerel (Bq)

1 becquerel = 1 decay/s = 1 Hz

(1 g Radium = 1 Ci)

D37:

D_{10} value = D_{37} / (–log (0.3679))

APPENDIX 3 – Additional tables

Table 7. Doses required to decrease selected pathogens at refrigerator temperatures by one decimal log/90% (D-dose)

Pathogens	D-dose in kGy*	5-log reduction dose in kGy
Campylobacter	0.2	1
Toxoplasma cysts	0.25	1.25
Escherichia coli O157	0.3	1.5
Listeria	0.45	2.25
Salmonella	0.7	3.5
Clostridium botulinum spores	3.6	18
*1 gray = 100 rads; 10 kGy = 1 Mrad		

Source: Robert V. Tauxe CDC, cdc.gov/ncidod/eid/vol7no3_supp/tauxe.htm, accessed 29 March 2011.

Table 8. Estimated ionising irradiation D_{10} values for viruses

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Adenoviridae		Adenovirus 12 (Eagle's MEM plus 2% FBS)	4.60	Sullivan et al (1971)
Adenoviridae		Adenovirus 2 (Eagle's MEM plus 2% FBS)	3.80–4.60	Sullivan et al. (1971)
Adenoviridae		Adenovirus 3 (Eagle's MEM plus 2% FBS)	4.90	Sullivan et al.(1971)
Adenoviridae		Adenovirus 5 (Eagle's MEM plus 2% FBS)	4.40	Sullivan et al.(1971)
Adenoviridae	Aviadenovirus	Avian adenovirus (cell culture medium)	4.50	Thomas et al. (1981)
Adenoviridae	Mastadenovirus	Canine adenovirus (FBS)	5.17–5.91	Invitrogen Corporation (2011)
Adenoviridae	Mastadenovirus	Canine adenovirus (FBS)	3.50	Sofer et al.(2003)
Arenaviridae	Arenavirus	Lassa (PBS/BSA or human serum at 4 °C)	1.90	Elliott et al.(1982)
Arenaviridae	Arenavirus	Lassa (PBS/BSA or human serum at -60 °C)	3.20	Elliott et al.(1982)
Arteriviridae	Arterivirus	Porcine reproductive and respiratory syndrome virus (glucose)	11.10–12.50	Purtle et al.(2006a)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Asfarviridae	Asfarvirus	African swine fever (whole swine blood)	<2.00	Thomas et al.(1981)
Birnaviridae	Avibirnavirus	Infectious bursal disease virus (phosphate buffered saline)	6.20	Jackwood et al. (2007)
Birnaviridae	Aquabirnavirus	Infectious pancreatic necrosis virus (salmonids)	10.00	Ahne (1982)
Bunyaviridae	Orthobunyavirus	Aino virus (bovine serum at -68 °C)	3.50	House et al. (1990)
Bunyaviridae	Orthobunyavirus	Akabane virus (cell culture medium)	<2.00	Thomas et al. (1981)
Bunyaviridae	Orthobunyavirus	Akabane virus (bovine serum at - 68 °C)	2.50	House et al. (1990)
Caliciviridae	Norovirus	Murine norovirus 1 (virus inoculated into fresh produce)	3.3	Feng et al. (2011)
Coronoviridae	Coronavirus	Transmissible gastroenteritis (cell culture medium)	<2.00	Thomas et al. (1981)
Coronoviridae	Coronavirus	Transmissible gastroenteritis (cell culture medium)	<3.10	Simon et al.(1983)
Coronoviridae	Coronavirus	Transmissible gastroenteritis (liquid manure)	<3.60	Simon et al.(1983)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Filoviridae	Ebolavirus	Ebola virus (PBS/ BSA or human serum at 4 ^{o}C)	1.50	Elliott et al. (1982)
Filoviridae	Ebolavirus	Ebola virus (PBS/ BSA or human serum at - 60 °C)	2.15	Elliott et al. (1982)
Filoviridae	Ebolavirus	Ebola virus	2.30	Lupton (1981)
Filoviridae	Marburgvirus	Marburg virus (PBS/ BSA or human serum at 4 ^{o}C)	1.20	Elliott et al.(1982)
Filoviridae	Marburgvirus	Marburg virus (PBS/ BSA or human serum at - 60 °C)	2.10	Elliott et al.(1982)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (in Factor V111)	4.50	Reid (1998)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (albumin)	8.30	Reid (1998)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (fibrinogen)	8.60	Reid (1998)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (FBS)	4.10	Daley et al.(1998)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (human albumin)	6.80	Miekka et al.(2003)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (equine serum)	4.10	Willkommen et al. (1999)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (frozen virus suspension)	<3.00	Pruss et al. (2001)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (in bone)	<5.20 to <6.40	Pruss et al. (2001)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (bovine serum)	4.10	Rojas et al. (2006)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (cell culture medium)	<2.00	Thomas et al. (1981)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (equine serum)	5 .00 (two data points)	Willkommen et al. (1999)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (FCS)	5.70	Hermann et al. (2005)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (cell culture medium)	<3.60	Simon et al. (1983)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (DHS/FBS)	5.10–8.10	Purtle et al. (2006b)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (liquid manure)	<3.10	Simon et al. (1983)
Flaviviridae	Pestivirus	Bovine viral diarrhoea virus (FBS)	3.68 - 5.10	Invitrogen Corporation (2011)
Flaviviridae	Pestivirus	Classical swine fever (bovine serum)	5.50	House et al. (1990)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Flaviviridae	Pestivirus	Classical swine fever	8.60	Preliminary data as quoted by pers.comm. Richmond to Callis, Plum Island Animal Disease Centre, US (1981)
Flaviviridae	Pestivirus	Classical swine fever (Brescia strain) (culture medium)	1.80	Groneman et al. (1977)
Flaviviridae	Flavivirus	St Louis encephalitis from brain (phosphate buffer)	5.81	Jordan and Kempe (1956)
Flaviviridae	Flavivirus	St Louis encephalitis crude virus (phosphate buffer)	6.20	Jordan and Kempe (1956)
Flaviviridae	Flavivirus	St Louis encephalitis partially purified virus (phosphate buffer)	3.87	Jordan and Kempe (1956
Flaviviridae	Pestivirus	Bovine virus diarrhoea virus (FBS/Donor horse serum)	5.10–8.10	Purtle et al. (2006b)
Flaviviridae	Flavivirus	Yellow fever virus (stabilisers (medium) and vero cells (substrate) at - 40 °C)	3.37	Kitchen et al. (1989)
Herpesviridae	Subfamily Alphaherpesvirinae, genus Varicellovirus	Bovine herpesvirus 1 (IBR) (FCS)	2.86	Willkommen et al. (1999)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Herpesviridae	Subfamily Alphaherpesvirinae, genus Varicellovirus	Bovine herpesvirus 1 (IBR) (cell culture medium)	<2.00	Thomas et al. (1981)
Herpesviridae	Subfamily Alphaherpesvirinae, genus Varicellovirus	Bovine herpesvirus 1 (IBR) (FCS)	4.92	Hermann et al. (2005)
Herpesviridae	Subfamily Alphaherpesvirinae, genus Varicellovirus	Bovine herpesvirus 1 (IBR)(FBS)	4.10	Hanson and Foster (1997)
Herpesviridae	Subfamily Alphaherpesvirinae, genus Varicellovirus	Bovine herpesvirus 1 (IBR) (DHS/FBS)	5.60–6.40	Purtle et al.(2006b)
Herpesviridae	Subfamily Alphaherpesvirinae, genus Varicellovirus	Bovine herpesvirus 1 (IBR) (FBS)	3.10–4.40	Invitrogen Corporation (2011)
Herpesviridae	Subfamily Alphaherpesvirinae, genus Varicellovirus	Bovine herpesvirus 1 (IBR)(FBS)	4.72–7.31	Degiorgi et al. (1999)
Herpesviridae	Subfamily Alphaherpesvirinae genus Simplexvirus	Herpes simplex virus (HSV 1 Ang) (Eagle basal medium + FCS in -80 °C in dry ice)	3.68	Rösen et al. (1987)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Herpesviridae	Subfamily Alphaherpesvirinae genus Simplexvirus	Herpes simplex virus (HSV 1 Kos) (Eagle basal medium + FCS in -80 °C in dry ice)	2.30	Rösen et al. (1987)
Herpesviridae	Subfamily Alphaherpesvirinae genus Simplexvirus	Herpes simplex virus (HSV 1 Muller) (Eagle basal medium + FCS in in -80 °C in dry ice)	3.68	Rösen et al. (1987)
Herpesviridae	Subfamily Alphaherpesvirinae genus Simplexvirus	Herpes simplex virus (HSV 1 Thea) (Eagle basal medium + FCS in in -80 °C in dry ice)	2.76	Rösen et al.(1987)
Herpesviridae	Subfamily Alphaherpesvirinae genus Simplexvirus	Herpes simplex virus (HSV) (Hank's solution)	2.58–3.08	Smolko and Lombardo (2005)
Herpesviridae	Subfamily Alphaherpesvirinae genus Simplexvirus	Herpes simplex virus (HSV) (Eagle's MEM plus 2% FBS)	3.90–4.60	Sullivan et al. (1971)
Herpesviridae	Subfamily Alphaherpesvirinae, genus Simplex virus	Herpes simplex virus (HSV-1) (medium and serum substrate vero cells at -40 °C	3.52	Kitchen et al.(1989)
Herpesviridae	Subfamily Alphaherpesvirinae genus Varicellovirus	Porcine herpesvirus 1 (Aujeszky's/pseudorabies) (cortical diaphysis at 30±5 °C)	5.30	Pruss et al. (2001)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Herpesviridae	Subfamily Alphaherpesvirinae genus Varicellovirus	Porcine herpesvirus 1 (Aujeszky's/pseudorabies) (in bone)	<6.50 to <7.00	Pruss et al. (2001)
Herpesviridae	Subfamily Alphaherpesvirinae genus Varicellovirus	Porcine herpesvirus 1 (Aujeszky's/pseudorabies) (cell culture medium)	<2.00	Thomas et al. (1981)
Herpesviridae	Subfamily Alphaherpesvirinae genus Varicellovirus	Porcine herpesvirus 1 (Aujeszky's/pseudorabies) (frozen cell culture suspension)	4.56	Sun et al. (1978)
Herpesviridae	Subfamily Alphaherpesvirinae genus Varicellovirus	Porcine herpesvirus 1 (Aujeszky's/pseudorabies)(cell culture media)	<2.86	Simon et al.(1983)
Herpesviridae	Subfamily Alphaherpesvirinae genus Varicellovirus	Porcine herpesvirus 1 (Aujeszky's/pseudorabies) (liquid swine manure)	<3.10	Simon et al.(1983)
Herpesviridae	Subfamily Alphaherpesvirinae genus Varicellovirus	Porcine herpesvirus 1 (Aujeszky's/pseudorabies) (in suspension)	1.47	Brodorotti (1978) as quoted by pers. comm. Richmond to Callis, Plum Island Animal Disease Centre, US (1981)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Herpesviridae	Subfamily Alphaherpesvirinae genus Varicellovirus	Porcine herpesvirus 1 (Aujeszky's/pseudorabies) (lyophilised)	4.67	Brodorotti (1978) as quoted by pers. comm. Richmond to Callis, Plum Island Animal Disease Centre US (1981)
Herpesviridae	Subfamily Alphaherpesvirinae, Genus Mardivirus	Turkey herpes virus (Sucrose-Phosphate-Glutamate-Albumin buffer and substrate chicken embryo fibroblasts)	5.10	Kitchen et al. (1989)
Orthomyxoviridae	Influenzavirus A	16 HA and 9 NA subtypes (water)	1.00	Sullivan et al. (1971)
Orthomyxoviridae	Influenzavirus A	Avian influenza (liquid chicken faeces)	0.70	Thomas et al. (1982)
Orthomyxoviridae	Influenzavirus A	Avian influenza A (Eagle's MEM plus 2% FBS)	4.60–4.90	Sullivan et al. (1971)
Orthomyxoviridae	Influenzavirus A	PR8 strain (H1N1) (10% FBS at - 40 °C) (PFU)	2.82	Lowy et al. (2001)
Orthomyxoviridae	Influenzavirus A	PR8 strain (H1N1) (10% FBS at - 40 °C) (TCID)	5.77	Lowy et al. (2001)
Orthomyxoviridae	Influenzavirus A	X31 strain (H3N2) (10% FBS at – 40 °C) (PFU)	2.46	Lowy et al. (2001)
Orthomyxoviridae	Influenzavirus A	X31 strain (H3N2) (10% FBS at – 40 °C) (TCID)	7.08	Lowy et al. (2001)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Paramyxoviridae	Subfamily Paramyxovirinae and genus Respirovirus	Bovine parainfluenza 3 (FBS)	4.10	Hanson and Foster (1997)
Paramyxoviridae	Subfamily Paramyxovirinae and genus Respirovirus	Bovine parainfluenza 3 (cattle, sheep, other mammals) (FCS)	3.75	Willkommen et al. (1999)
Paramyxoviridae	Subfamily Paramyxovirinae and genus Respirovirus	Bovine parainfluenza 3 (cattle, sheep, other mammals) (FCS)	4.67	Hermann et al. (2005)
Paramyxoviridae	Subfamily Paramyxovirinae and genus Morbillivirus	Measles virus (Aliquots of measles virus sealed in glass ampoules)	0.65	Musser et al. (1960)
Paramyxoviridae	Subfamily Paramyxovirinae and genus Morbillivirus	Measles virus (stabilizers not specified)	4.10	Kitchen et al. (1989)
Paramyxoviridae	Subfamily Paramyxovirinae genus Avulavirus	Newcastle disease (Eagle's MEM plus 2% FBS)	5.20	Sullivan et al. (1971)
Paramyxoviridae	Subfamily Paramyxovirinae genus Avulavirus	Newcastle disease (Egg fluid)	2.00	Thomas et al. (1981)
Paramyxoviridae	Subfamily Paramyxovirinae genus Avulavirus	Newcastle disease (infective allantois fluid)	1.71	DiGioia et al.(1970)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Paramyxoviridae	Subfamily Paramyxovirinae genus Avulavirus	Newcastle disease	2.58	Brodorotti (1978)as quoted by pers. comm Richmond to Callis, Plum Island Animal Disease Centre, US (1981)
Paramyxoviridae	Subfamily Paramyxovirinae and genus Morbillivirus	Rinderpest virus (PBS)	1.80	Saliki et al. (1993)
Parvoviridae	Betaparvovirus	Bovine parvovirus (in bone at – 30±5^{o}C)	7.40–10.10	Pruss et al. (2001)
Parvoviridae	Betaparvovirus	Bovine parvovirus (frozen virus suspensions in plastic tubes at – 30±5 °C)	7.30	Pruss et al. (2001)
Parvoviridae	Betaparvovirus	Bovine parvovirus (FCS)	19.44	Hermann et al .(2005)
Parvoviridae	Parvovirus	Feline panleucopenia virus	7.63	Kitchen et al. (1989)
Parvoviridae	Parvovirus	Minute virus of mice (bovine serum)	6.57–9.21	Purtle et al. (2006b)
Parvoviridae	Parvovirus	Minute virus of mice	10.70	House et al.(1990)
Parvoviridae	Parvovirus	Porcine parvovirus (FBS)	5.00–15.00	Hanson and Foster (1997)
Parvoviridae	Parvovirus	Porcine parvovirus (25% human albumin)	5.29–6.85	Miekka et al. (2003)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Parvoviridae	Parvovirus	Porcine parvovirus (FCS)	21.05	Willkommen et al (1999)
Parvoviridae	Parvovirus	Porcine parvovirus (pulverized bone)	9.40	Grieb et al. (2005)
Parvoviridae	Parvovirus	Porcine parvovirus(cell culture fluid)	4.00	Thomas et al. (1981)
Parvoviridae	Parvovirus	Porcine parvovirus (anti-insulin monoclonal anitibody+bovine serum +albumin)	10.00	Grieb et al. (2005)
Parvoviridae	Parvovirus	Porcine parvovirus (pulverised bone)	9.60	Grieb et al. (2005)
Parvoviridae	Parvovirus	Porcine parvovirus (Factor VIII)	6.30	Reid (1998)
Parvoviridae	Parvovirus	Porcine parvovirus (albumin)	6.70	Reid (1998)
Parvoviridae	Parvovirus	Porcine parvovirus (Fibrinogen)	7.00	Reid (1998)
Parvoviridae	Parvovirus	Porcine parvovirus (Glucose)	15.50	Purtle et al. (2006a)
Parvoviridae	Parvovirus	Porcine parvovirus (Alpha-1-proteinase inhibitor)	6.75	Reid (1998)
Parvoviridae	Parvovirus	Porcine parvovirus (FBS)	<3.50 to<4.90	Invitrogen Corporation (2011)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Picornaviridae	Teschovirus	Porcine enterovirus 1 (Teschen disease) (cell culture medium)	2.00	Simon et al.(1983)
Picornaviridae	Enterovirus	Coxsackievirus A-11 (Eagle's MEM plus 2% FBS)	4.80	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Coxsackievirus A-9 (Eagle's MEM plus 2% FBS)	4.20–4.60	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Coxsackievirus A-9 (in water)	1.20	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Coxsackievirus B-1(Eagle's MEM plus 2% FBS)	4.10	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Coxsackievirus B-2	4.40–4.50	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Coxsackievirus B-2 (in water)	1.40	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Coxsackievirus B-3 (Eagle's MEM plus 2% FBS)	3.5–4.3	Sullivan et al. (1971)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Picornaviridae	Enterovirus	Coxsackievirus B-4 (Eagle's MEM plus 2% FBS)	5.00	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Coxsackievirus (in frozen food)	6.8–8.1	Farkas (2007)
Picornaviridae	Enterovirus	Coxsackievirus B-5 (Eagle's MEM plus 2% FBS)	4.10	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Echovirus 11 (Eagle's MEM plus 2% FBS)	4.2–4.8	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Echovirus 11 (in water)	1.40	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Echovirus 12 (Eagle's MEM plus 2% FBS)	5.00	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Echovirus 18 (Eagle's MEM plus 2% FBS)	4.40	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Echovirus 4 (Eagle's MEM plus 2% FBS)	4.60	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Echovirus 5 (Eagle's MEM plus 2% FBS)	4.90	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Echovirus 6 (Eagle's MEM plus 2% FBS)	5.10	Sullivan et al. (1971)
Picornaviridae	Enterovirus	Echovirus 7 (Eagle's MEM plus 2% FBS)	3.8–5.7	Sullivan et al. (1971)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Picornaviridae	Enterovirus	Echovirus 9 (Eagle's MEM plus 2% FBS)	5.00	Sullivan et al. (1971)
Picornaviridae	Cardiovirus	Encephalomyocarditis virus (25% human albumin)	3.20	Miekka et al.(2003)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type O in liquid state	4.60	Massa (1966) from Baldelli et al. (1965)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type A in liquid state	4.80	Massa (1966) from Baldelli et al. (1965)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type C in liquid state	4.80	Massa (1966) from Baldelli et al. (1965)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type C in liquid state	5.00	Massa (1966) from Baldelli et al. (1965)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virusType C in dry state	6.70	Massa (1966) from (Baldelli et al. (1965)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type O in bone marrow	3.50	Massa (1966) from Baldelli et al. (1965)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type O in blood	3.50	Massa (1966) from Baldelli et al. (1965)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type O in lymph glands	3.50	Massa (1966) from Baldelli et al. (1965)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type A in blood	5.00	Massa (1966) from Baldelli et al. (1965)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type C in blood	4.70	Massa (1966) from Baldelli et al. (1965) (Baldelli et al. 1965)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type O in blood	4.70	Massa (1966) from Baldelli et al. (1965)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus (Hanks' salt solution with 0.5 lactalbumin hydrolyzate and 2% bovine serum)	5.00	Polatnick and Bachrach (1968)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus (bovine serum)	5.30	House et al.(1990)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type C (cell culture medium)	4.1–4.3	Simon et al.(1983)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type C in liquid manure	6.00	Simon et al.(1983)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type Types A, O & C (Hank's solution)	6.20	Smolko and Lombardo (2005)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus Type A (air dried virus)	6.50	Dekker (1998)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus (type O-1) (Frenkel culture medium+ 2 M NaBr)	6.10	Groneman et al.(1977)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus (type O-1) (Frenkle culture medium)	6.50	Groneman et al.(1977)
Picornaviridae	Aphtovirus	Foot-and-mouth disease virus (type O-1) (BHK culture medium and Frenkel culture medium)	4.30–4.70	Groneman et al.(1977)
Picornaviridae	Hepatovirus	Hepatitis A (frozen virus in plastic bottles)	5.30	Pruss et al.(2001)
Picornaviridae	Hepatovirus	Hepatitis A (in bone)	<4.60 to <4.70	Pruss et al.(2001)
Picornaviridae	Hepatovirus	Hepatitis A (fruit and vegetables)	2.72–2.97	Bidawid et al.(2000)
Picornaviridae	Hepatovirus	Hepatitis A (lettuce /strawberries)	2.67–3.63	Sattar et al. (2000)
Picornaviridae	Enterovirus	Human coxsackieviruses (Eagle's MEM)	5.90–6.90	Sullivan et al.(1973)
Picornaviridae	Enterovirus	Human coxsackieviruses (distilled water)	5.30	Sullivan et al.(1973)
Picornaviridae	Enterovirus	Human coxsackieviruses (cooked ground beef)	6.80–8.10	Sullivan et al.(1973)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Picornaviridae	Enterovirus	Human coxsackieviruses (raw ground beef)	6.80–7.50	Sullivan et al.(1973)
Picornaviridae	Enterovirus	Poliomyelitis virus-1	3.10	Kitchen et al.(1989)
Picornaviridae	Enterovirus	Polio-virus 1	4.00	Girolamo et al. (1972)
Picornaviridae	Enterovirus	Poliovirus (Ab Sabin strain) (PIS) (Eagle's basal medium with Hank's balanced salt solution)	6.00	Heidelbaugh and Giron (1969)
Picornaviridae	Enterovirus	Poliovirus (III-Leon strain) (Eagle's MEM plus 2% FBS)	3.80–4.80	Sullivan et al. (1973)
Picornaviridae	Enterovirus	Poliovirus (III-Leon strain) (in water)	4.10–5.40	Sullivan et al.(1973)
Picornaviridae	Enterovirus	Poliovirus (III-Nadler strain) (Eagle's MEM plus 2% FBS)	4.80	Sullivan et al.(1973)
Picornaviridae	Enterovirus	Poliovirus (II-Lansing strain) (Eagle's MEM plus 2% FBS)	4.10–5.00	Sullivan et al.(1973)
Picornaviridae	Enterovirus	Poliovirus (II-Y-SK strain) (Eagle's MEM plus 2% FBS)	4.10–5.40	Sullivan et al.(1973)
Picornaviridae	Enterovirus	Poliovirus (I-Lotshaw strain) (Eagle's MEM plus 2% FBS)	5.30	Sullivan et al.(1973)
Picornaviridae	Enterovirus	Poliovirus (I-MAH strain) (Eagle's MEM plus 2% FBS)	4.90	Sullivan et al.(1973)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Picornaviridae	Enterovirus	Poliovirus (Lansing strain) in brain (phosphate buffer)	7.57	Jordan and Kempe (1956)
Picornaviridae	Enterovirus	Poliovirus (Lansing strain) crude virus (phosphate buffer)	4.65	Jordan and Kempe (1956)
Picornaviridae	Enterovirus	Poliovirus (Lansing strain) partially purified virus (phosphate buffer)	6.20	Jordan and Kempe (1956)
Picornaviridae	Enterovirus	Poliovirus (PV-1) (Frozen virus suspensions in plastic tubes)	7.10	Pruss et al. (2001)
Picornaviridae	Enterovirus	Poliovirus (PV-1) (in bone)	4.40–5.20	Pruss et al. (2001)
Picornaviridae	Enterovirus	Poliovirus (Sabin 1 vaccine strain)	4.90	Kitchen et al. (1989)
Picornaviridae	Enterovirus	Swine vesicular disease (cell culture medium)	5.50	Thomas et al. (1981)
Picornaviridae	Enterovirus	Swine vesicular disease (chopped tissue)	5.40	Thomas et al. (1982)
Picornaviridae	Enterovirus	Swine vesicular disease (frozen chopped lymph node)	4.80	Thomas et al. (1982)
Picornaviridae	Enterovirus	Swine vesicular disease (cell culture medium and cellular debris, mixed wet with sewage sludge)	3.80	Thomas et al. (1982)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Picornaviridae	Enterovirus	Swine vesicular disease (bovine serum)	5.00	House et al. (1990)
Picornaviridae	Enterovirus	Swine vesicular disease (cell culture medium)	4.00–5.00	Simon et al. (1983)
Picornaviridae	Enterovirus	Swine vesicular disease (liquid manure)	3.85	Simon et al. (1983)
Picornaviridae	Enterovirus	Swine vesicular disease (England 72 strain) (culture media + raw sludge+0.2 M NaBr)	5.90	Groneman et al. (1977)
Picornaviridae	Enterovirus	Swine vesicular disease (England 72 strain) (culture media + raw sludge)	6.20	Groneman et al. (1977)
Polyomaviridae	Polyomavirus	Polyoma virus (Eagle's MEM)	12.00	Basilico and di Mayorca (1965)
Polyomaviridae	Polyomavirus	Simian virus 40 (Eagle's MEM plus 2% FBS)	3.90–4.50	Sullivan et al.(1971)
Poxviridae		Avian pox (cell culture medium)	2.20	Thomas et al.(1981)
Poxviridae		Avian pox	1.87	Brodorotti (1978) as quoted by pers. comm. Richmond to Callis, Plum Island Animal Disease Centre, US (1981)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Poxviridae	Subfamily Chordopoxvirinae, genus Orthopoxvirus	Vaccinia (Armstrong strain) in brain	4.47	Jordan and Kempe (1956)
Poxviridae	Subfamily Chordopoxvirinae, genus Orthopoxvirus	Vaccinia (Armstrong strain) crude virus (phosphate buffer)	3.88	Jordan and Kempe (1956)
Poxviridae	Subfamily Chordopoxvirinae, genus Orthopoxvirus	Vaccinia (Armstrong strain) partially purified virus (phosphate buffer)	2.35	Jordan and Kempe (1956)
Poxviridae	Subfamily Chordopoxvirinae, genus Orthopoxvirus	Vaccinia (Copenhagen strain) (25% solution human albumin)	2.07	Miekka et al.(2003)
Poxviridae	Subfamily Chordopoxvirinae, genus Orthopoxvirus	Vaccinia (IHD strain)	4.18	Friesen (1963) as quoted by Lowy (2005)
Poxviridae	Subfamily Chordopoxvirinae, genus Orthopoxvirus	Vaccinia (Lancy strain) (Chorio-allontoic dilution)	8.14	Palacios (1963) as quoted by Lowy (2005)
Poxviridae	Subfamily Chordopoxvirinae, genus Orthopoxvirus	Vaccinia (Levanditi strain) (PBS)	2.62	Wilson (1961) as quoted by Lowy (2005)
Poxviridae	Subfamily Chordopoxvirinae, genus Orthopoxvirus	Vaccinia (Merieux 37 (Lister) strain) (10% calf serum)	1.70	Decker et al.(1969)
Poxviridae	Subfamily Chordopoxvirinae, genus Orthopoxvirus	Vaccinia (Rabbit strain)	5.25	Lea (1942) as quoted by Decker et al.(2005)
Reoviridae	Orbivirus	African horse sickness (Equine) (cell culture at 4 °C)	1.70	Thomas and Samagh (1984)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Reoviridae	Orbivirus	African horse sickness (Equine) (cell culture at - 190 °C)	3.40	Thomas and Samagh (1984)
Reoviridae	Orbivirus	African horse sickness (Equine) (mouse brain at 4 °C)	1.90	Thomas and Samagh (1984)
Reoviridae	Orbivirus	African horse sickness (Equine) (mouse brain at- 190 °C)	4.80	Thomas and Samagh (1984)
Reoviridae	Orbivirus	Bluetongue virus (whole bovine blood)	<2.00	Thomas et al. (1981)
Reoviridae	Orbivirus	Bluetongue virus (whole sheep blood)	1.00	Thomas et al. (1982)
Reoviridae	Orbivirus	Bluetongue virus	6.58 (only 2 data points)	Willkommen et al. (1999)
Reoviridae	Orbivirus	Bluetongue virus (bovine serum)	8.30	House et al.(1990)
Reoviridae	Orbivirus	Bluetongue virus(cell culture at 4 °C)	<2.00	Thomas and Samagh (1984)
Reoviridae	Orbivirus	Bluetongue virus (cell culture at – 190 °C)	1.10	Thomas and Samagh (1984)
Reoviridae	Orbivirus	Bluetongue virus inmouse brain (at 4 °C)	4.30	Thomas and Samagh (1984)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Reoviridae	Orbivirus	Bluetongue virus (mouse brain at – 190 °C)	2.00	Thomas and Samagh (1984)
Reoviridae	Orbivirus	Bluetongue virus (FBS)	7.50–10.50	Purtle et al. (2006a)
Reoviridae	Orbivirus	Bluetongue virus (FBS)	6.25–7.10	Hanson and Foster (1997)
Reoviridae	Orthoreovirus	Reovirus (FCS)	3.73	Willkommen et al. (1999)
Reoviridae	Orthoreovirus	Reovirus	4.00	Brodorotti (1978) as quoted by pers. comm. Richmond to Callis, Plum Island Animal Disease Centre, US (1981)
Reoviridae	Orthoreovirus	Reovirus (Reo-3) (FBS)	3.57–4.48	Invitrogen Corporation (2011)
Reoviridae	Orthoreovirus	Reovirus (Reo-3) (FCS)	5.20	Hermann et al.(2005)
Reoviridae	Orthoreovirus	Reovirus 1 (Eagle's MEM plus 2% FBS)	4.20–4.40	Sullivan et al.(1971)
Reoviridae	Orthoreovirus	Reovirus (FBS)	4.16	Hanson and Foster (1997)
Retroviridae	Gammaretrovirus	Feline leukemia virus (bovine serum)	7.59–10.6	Purtle et al.(2006b)
Retroviridae	Lentivirus	Human immunodeficiency virus (FCS).	8.80	Campbell and Li (1999)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Retroviridae	Lentivirus	Human immunodeficiency virus	6.30	Kitchen et al. (1989)
Retroviridae	Lentivirus	Human immunodeficiency virus (Frozen virus suspensions in plastic tubes)	7.10	Pruss et al. (2001)
Retroviridae	Lentivirus	Human immunodeficiency virus (in bone)	<8.30 to<8.90	Pruss et al. (2001)
Retroviridae	Lentivirus	Maedi visna virus (cell culture medium)	3.50	Thomas et al. (1981)
Retroviridae	Gammaretrovirus	Murine leukemia virus	1.31–4.78	Smolko and Lombardo (2005)
Rhabdoviridae	Ephemerovirus	Bovine ephemeral fever virus (bovine serum at - 68 °C)	2.90	House et al. (1990)
Rhabdoviridae	Lyssavirus	Rabies (CVS-11 strain) (brain)	2.30	Gamble et al. (1980)
Rhabdoviridae	Lyssavirus	Rabies (CVS-11 strain)	2.70	Gamble et al. (1980)
Rhabdoviridae	Vesiculovirus	Vesicular stomatitis	2.00	Thomas et al. (1982)
Togaviridae	Alphavirus	Sindbis (pulverised bone at or below - 65 °C)	10.20	Grieb et al. (2005)
Togaviridae	Alphavirus	Venezuelan equine encephalitis	6.04–7.15	Reitman and Tribble, Jr.(1967)

Family	Genus	Species (Media)	Estimated / calculated D_{10} (kGy)	Reference
Togaviridae	Alphavirus	Venezuelan equine encephalitis	4.57	Pers. comm. Smith J (Virology Division, USAMRIID) to Murray K (AAHL) (1990)
Togaviridae	Alphavirus	Western equine encephalitis (in brain)	5.17	Jordan and Kempe (1956)
Togaviridae	Alphavirus	Western equine encephalitis (partially purified virus in phosphate buffer)	3.87	Jordan and Kempe (1956)
Togaviridae	Alphavirus	Western equine encephalitis (crude virus in phosphate buffer)	5.43	Jordan and Kempe (1956)

Table 9. Estimated ionising irradiation D_{10} values for bacteria

Name of Bacteria	D10 (kGy)
Acholeplasma laidlawii	1.16 - 1.78
Achromobacter aquamarinus	0.08
Achromobacter pen sensitive	0. 4
Achromobacter sp.	0. 1
Acinetobacter anitratus	0.2
Acinetobacter calcoaceticus	0.06 - 8.14
Aerobacter aerogenes	1.00
Aeromonas hydrophila	0.06
Bacillus anthracis	2.4
Bacillus brevis	0.5
Bacillus cereus	0.12 - 3.8
Bacillus globigii	1.0 - 3.5
Bacillus licheniformis	1.82
Bacillus megaterium	0.25 - 4.0
Bacillus mesentericus	1.18
Bacillus pumilis	0.16 - 3.8
Bacillus sphaericus	1.5 - 7.8
Bacillus stearothermophilus	0.94 - 9.0
Bacillus subtilis	0.12 - 5.0
Bacillus thermoacidurans	1.2
Brevibacterium sp.	4.85 - 6.42
Brucella abortus	0.035
Campylobacter jejuni	0.08 - 0.32
Clostridium aerofoetidum	1.6
Clostridium bifermentans	1.4 - 2.0
Clostridium botulinum type A	1.0 - 2.7
Clostridium boltulinum type B	1.0 - 2.28
Clostridium botulinum type C	1.4 - 1.63
Clostridium botulinum type D	2.2
Clostridium botulinum type E	0.8 - 1.31
Clostridium botulinum type F	2.5
Clostridium butyricum	1.5
Clostridium caloritolerans	1.5
Clostridium chauvoei	2.0
Clostridium fullax	2.5
Clostridium histolyticum	1.8 - 10
Clostridium oedematiens type C	1.6
Clostridium oedematiens type A	1.9
Clostridium oedematiens type B	1.8
Clostridium perfringens	1.32 - 2.88
Clostridium septicum	1.6
Clostridium septricum	7.5
Clostridium sordellii	1.5
Clostridium sphenoides	2.1
Clostridium sporogenes	0.6 - 6.2
Clostridium subterminale	1.6
Clostridium tetani	1.77 - 5.0
Clostridium tetanomorphum	1.8 - 2.3
Clostridium tortillion	1.6
Clostridium welchii type A	1.2
Clostridium welchii type B	1.7
Clostridium welchii type C	1.8
Clostridium welchii type E	1.2

Name of Bacteria	D10 (kGy)
Clostridium welchii type F	2.0
Corynebacterium	7.5
E coli K99	0.02
E coli	0.04 - 0.70
Enterobacter sp.	0.36 - 1.5
Flavobacterium sps	1.1
Klebsiella species	0.4 - 0.45
Lactobacillus brevio	0.12
Micrococcus cryophilus	1.6
Micrococcus luteus	1.1-1.2
Micrococcus pyogenes	0. 16 - 0. 44
Mycobacterium tuberculosis (BCG)	0. 35 - 1.28
Mycobacterium paratuberculosis	0.06
Nocardia sp.	6.5
Mycoplasma orale	3.9 - 5.15
Mycoplasma pneumoniae	3.76 - 4.92
Mycoplasma hyorhinis	4.13 - 5.39
Proteus mirabilis	0.24 - 0.5
Proteus vulgaris	0. 07
Pseudomonas aeruginosa	0. 13 - 0. 17
Pseudomonas fluorescens	0. 12
Pseudomonas maltophilia	0.18
Pseudomonas pyocyanea	0.2 - 1.4
Pseudomonas radiora	0.3 - 1.6
Pseudomonas sp.	0.03 - 0.19
Salmonella anatum	0.45 - 0.67
Salmonella dublin	0.02
Salmonella Enteriditis	0.25 - 0.7
Salmonella Gallinarum	0.13 - 0.56
Salmonella Heidelberg	0.33
Salmonella Meleagridis	0.93
Salmonella Panama	0.41 - 0.66
Salmonella Paratyphi	0.19 - 1.07
Salmonella Senftenberg	0.13 - 1.34
Salmonella Stanley	0. 61 - 0. 78
Salmonella Typhi	0. 2 - 0.78
Salmonella Typhimurium	0.1 - 1.58
Serratia marcescens	0.02 - 0.1
Staphylococcus albus	0.6
Staphylococcus aureus	0.13 - 2.6
Staphylococcus faecalis	0.63
Staphylococcus pathogenesis	1.0
Streptococcus faecalis	0. 01 - 8.7
Streptococcus faecium	0.06 - 9.2
Streptococcus viridans	1.4
Yersinia enterolitica	0.04 - 0.38

Note: The D_{10} ranges presented here were consolidated from various research, commercial and government sources. There is considerable variation in results between studies. Sources of information for any specific species are available on request.

APPENDIX 4 – References

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