Irradiation and Research Diets

SDS Info-Sheet 050523

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FURTHER INFORMATION

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OTHER SOURCES OF INFORMATION

www.isotron.com

Food Irradiation; A technique for preserving and improving the safety of food. WHO 1988 (Reprinted1991).
ISBN 92 4 154240 3.

1 Introduction

Sometimes it is the nature of research, or the animals being used which are sensitive to the normal and opportunistic micro-organisms that can be found in diets. Diet irradiation is a technique used to remove microbiological organisms which are inherently part of a diet.

2 What is 'Irradiation' and how does it basically work?

Irradiation is a broad term, which in the context of food 'sterilisation' is used to describe a form of short wavelength electromagnetic energy (Gamma rays), similar to Ultra Violet (UV). These waves of energy can penetrate a medium (diet) disrupting the normal molecular structure including DNA. This disruption then inhibits cellular growth (i.e. inhibits biological replication) and so decontaminates the product under exposure.

3 What is Gamma Irradiation and where does it come from?

Gamma Irradiation is the specific type of irradiation used to irradiate animal diets.

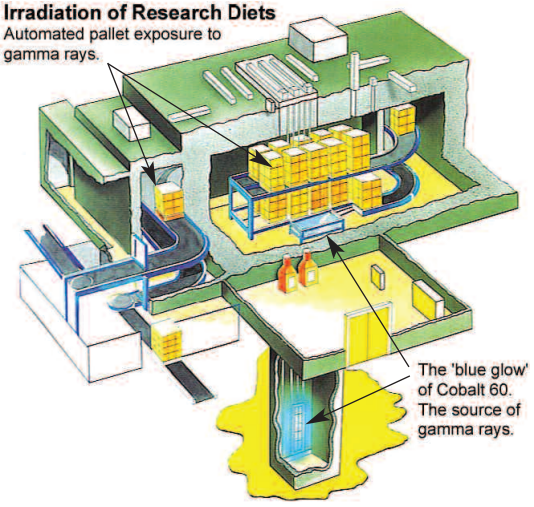
The source of Gamma rays used by SDS's contracted irradiation plant (ISOTRON), are from the radioisotope Cobalt 60. Co60 is an isotope of the stable Co59 and is produced in a nuclear reactor by bombarding Co59 with neutrons.

The decay of each atom of Co60 results in 2 photons of gamma radiation, a beta particle (which is captured immediately in the steel housing of the Co60) and a stable Nickel atom.

It is the photons of gamma irradiation that do the work by inducing molecular breaks via the creation of highly active electrons and free radicals in the target material.

4 What is the basic procedure for irradiating diet?

Each pallet is pre-programmed to receive a certain exposure (time x source activity). Pallets of product are loaded onto a conveyor where they are then moved around the gamma ray source allowing exposure to all sides of the product. Please see below.



The irradiation cell is a 2 meter thick concrete shield. The density of the concrete prevents gamma irradiation penetrating through to the outside world. The picture above shows the Cobalt 60 lowered in 5-6 meters of water - the safe position. This allows physical inspection within the cell. In the operational position the Cobalt 60 is raised to the centre of the irradiation cell.

5 How are irradiation exposures and dose levels measured?

The absorbed dose of irradiation is measured in Grays (Gy).
1 Gray = 1 joule of energy per kg of product.

Typical 'sterilising' doses range from 9 - 50 kilo Grays (kGy) *(See below for info. on levels required).*

To convert from the old system of measuring dose:

1 kilo Gray = 0.1 Mega Radiation Absorbed Dose (rad).
So 25 kGy = 2.5 Mega rad.

Absorbed dose (kGy) is dependant on the source (Co60) energy activity and the exposure period.

The source activity is carefully monitored because as Co60 decays, its activity is reduced by ~ 1% per month.

The exposure period is the main variable which is used to control the absorbed dose. It is the dwell time which is adjusted and monitored to control the dose.

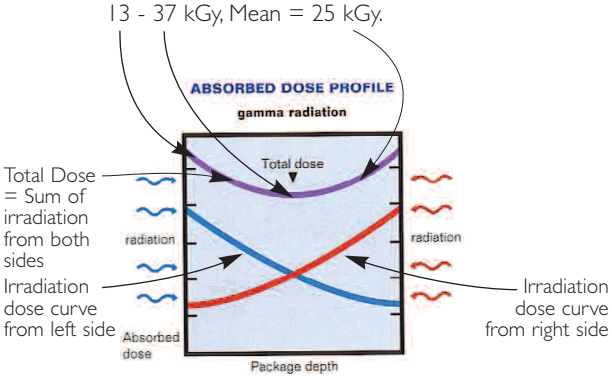
i.e. To increase the dose from 10 kGy to 25 kGy the product must be exposed to the source for 2.5 times the dwell period as a 10 kGy dosed product.

So, to increase the dose, a simple mathematical concept of increasing the units of exposure time can be applied. e.g. To attain a 50 kGy dose, a product may be sent through the conveyor system twice on a 25 kGy exposure routine.

Dose distribution and gamma ray penetration

Gamma rays are able to penetrate relatively dense products (but not 2 m of concrete). However, the absorbed dose of any particular kg of diet is dependant upon the position and distance in relation to the source. Although both sides of the pallet are exposed to the source (see below), it is the nature of the system that there will not be one constant absorbed dose though out every kg of diet - there will be a dose distribution.

An example of a dose distribution might be 13 - 37 kGy, Mean = 25 kGy.



The average dose is the sum of the minimum and maximum divided by 2.

Measuring and validating absorbed dose

Dose Mapping is a process used to characterise the dose distribution throughout a product. The process involves placing a matrix of dosimeters (coloured perspex discs) throughout a product and then measuring the change in colour (optical density) very accurately. The data obtained establishes the maximum, minimum and mean dose and importantly, the exposure time required to achieve a specified dose.

Routine indication of irradiation is carried out by placing colour changing 'detex' stickers on the product labels. These work in a similar way to dosimeters but only give a positive or negative indication of irradiation exposure. They cannot be used to measure dose.

6 What dose levels are required to 'sterilise' diets?

Sterilisation tends to infer that zero micro organisms remain following irradiation exposure. However, as is often in science, it is not that simple.

The reduction in viable organisms following exposure depends upon initial bioburden (microbiological loading) of the product, bioburden resistance to irradiation and absorbed dose. It is generally accepted that 25 kGy will sufficiently reduce the bioburden to acceptable levels for most research, without significantly affecting the product and its nutrition. 9 - 10 kGy is also frequently used.

It would be incorrect to suggest that there will be zero viable micro-organisms at these dose levels.

The laboratory limit of detection (LOD) for analysis of micro-organisms which often ranges from 1 - 10 colony forming units (cfu)/g, must also be taken into account.

Indication of dose levels and applications/affects on microbiology

Dose kGy	Application/Response
0.004	Lethal dose for humans.
0.01-0.04	Seeds of crops are mutated but may germinate.
0.05	Adult weevils made infertile.
0.05-0.15	Potato tubers killed.
0.2-0.3	Larvae and pupae of weevils inactivated.
0.4-0.8	Seeds of crops are killed.
1	General insect dis-infestation (U.S. FDA).
1.0-2.0	Many food spoilage organisms killed.
2.0-3.0	Some Salmonella spp. killed. Spores of some fruit spoilage fungi killed.
3.0-5.0	Most Salmonella spp. killed.
6	Proposed dose for peanuts/wheat grains. Over kill for seeds, but insufficient to eliminate spores of mycotoxing producing fungi.
10	General dose limit for foodstuffs. **9 - 10 kGy Used in some Research Diet situations.**
25	**Most commonly accepted dose for Research Diets being used inside barrier units.**
30	Suggested dose limit for herbs and spices.
50	Spores of food poisoning microbes such as Clostridium botulinum killed. **Used where improved Research Diet sterilisation is necessary - such as for use with gnotobiotic/germ free animals.**

The relationship between dose and reduction in the number of a viable organism (sensitivity) is quite precise although complex. More detailed information on individual micro-organisms sensitivity is available in the literature. For further information see: www.isotron.com/home.htm / Interesting Facts and Figures / D-10 values of micro organisms. The tables 1, 2, 3 and 4 show the dose required to reduce the micro-organism by level by 10^6, or 99.9999%. This level of reduction is often referred to as medical sterility.

Example;

On the whole, 5 kGy is sufficient to reduce many organisms by10^6 (6 decimal places).

This means 0.833 kGy is sufficient to reduce many organisms initial bioburden by 1 decimal place.

Minimum absorbed doses are ~ 13 kGy (for a 25 kGy Mean Dose).

So, 13/0.833 = 15.6 decimal reductions can be expected as a minimum.

Most SDS diets have very low initial bioburdens, but if you imagine a pre irradiation level of 10,000 Total viable organisms, 15.6 decimal reductions would reduce the initial bioburden to 2.5*10-12 cfu/g.

This level is way below the LOD and so would be considered 'sterile'.

For the mathematically minded, more detailed calculations and examples can be found at the back of this document.

7 Will the diet be radioactive?

Inducing radioactivity in materials exposed to irradiation is not possible with the Co60 gamma irradiation or high energy electrons up to energy levels of 5MeV. This applies to most materials up to an energy level of 15 MeV.

The 2 photons produced by each decaying Co60 atom are measured at 1.17 and 1.33 MeV respectively.

There is no hazardous residual radioactivity in materials irradiated with Co60.

8 What happens to nutrients?

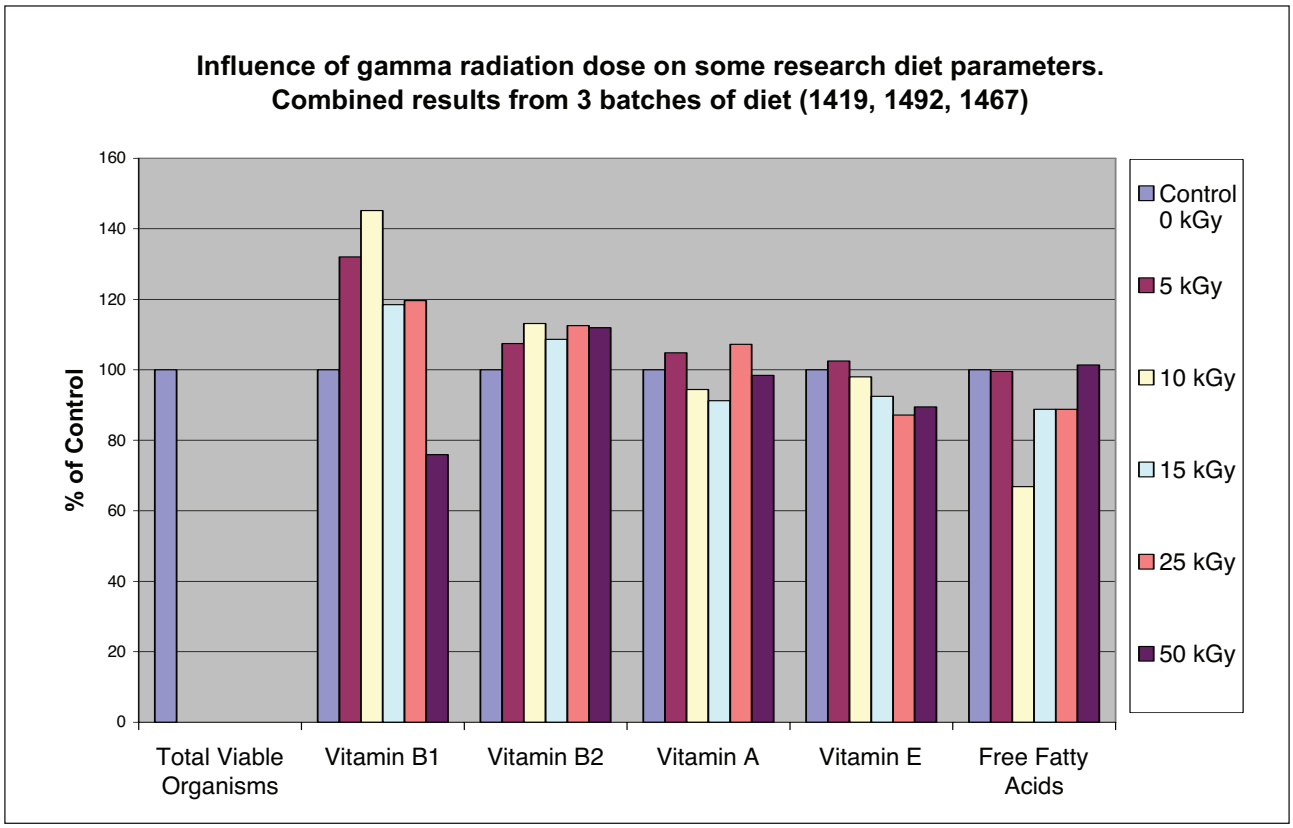
Because gamma rays are able to disrupt molecules, there is potential for degradation of essential nutrients.

However, the small number of molecular changes required to alter DNA sufficiently to inhibit growth/replication of micro-organisms are generally insufficient to cause significant damage to the nutrition of the product.

i.e. the doses used to destroy bacteria don't have much impact on the nutritional integrity of the diet.

Vitamins C, B1, E and A are the most sensitive vitamins. Long chain polyunsaturated fatty acids and sulphur containing amino acids can also be affected (Kilcast 1994).

SDS has conducted its own analysis on some of the pertinent nutrients over a range of gamma ray doses. For further information please see the chart (study 950808) below:



Notes: Vitamin C was not analysed in these rodent diets because it is only of importance to Guinea pigs and primates, and none is added to the diets in this study.

TVO – No detectable levels of TVO were found after being irradiated at any dose.

Vit. B1 – B1 analysis is notoriously variable. Although this is reported to be one of the most sensitive vitamins there is no degradation at least until the 50 kGy mean dose.

Vit. B2 – No significant difference following irradiation at any level.

Vit. A – Although this is reported to be one of the most sensitive vitamins there appears to be little degradation (<10% Maximum).

Vit. E – Up to 15% reduction appears to occur at mean doses of up to 50 kGy.

Free Fatty Acids – Although there is no significant difference, there is no degradation (i.e. increase) to the FFA level.

9 Appendix 1
Aid to understanding reduction in organisms following irradiation

% Reduction	Decimal place reduction	Fraction of original organism number remaining	Initial Bioburden Multiplication Factor	Example: Initial Bioburden TVO 50,000 cfu/g - Expected Post Irradiation cfu/g
0%	0	1/1	1	50,000
***90%**	**1**	**1/10**	**0.1**	**5,000**
99%	2	1/100	0.01	500
99.9%	3	1/1,000	0.001	50
99.99%	**4**	**1/10,000**	**0.0001**	**5 *****
99.999%	5	1/100,000	0.00001	0.5
****99.9999%**	**6**	**1/1,000,000**	**0.000001**	**0.05**
99.99999%	7	1/10,000,000	0.0000001	0.005
99.999999%	8	1/100,000,000	0.00000001	0.0005
99.9999999%	9	1/1,000,000,000	0.000000001	0.00005
99.99999999%	10	1/10,000,000,000	0.0000000001	0.000005

Often the level of decimal place reduction is many more times the levels shown here.

*90% Reduction is also known as the D10 figure.

**Reduction to 1 in a million is often referred to as medical sterility.

*** In this example, at a 4 decimal place reduction the expected level is below the Limit of Detection (LOD) for TVO.

10 Appendix 2
Calculation examples; dose vs microbiological affect

EXAMPLE 1

Normal sensitivity, low bioburden, normal dose
Expected post irradiated bioburden using dose, published sensitivity figures and initial bioburden.

Known information

1 Initial bioburden of micro organism i.e. Salmonella typhimurium of 100 cfu/g
(S.typhimurium is highly unlikely to be found in a research diet but makes a good 'normal sensitivity' example)

2 S.typhimurium dose sensitivity of <5.0 kGy for a 10^6 (6 decimal place) reduction in numbers.
(See www.isotron.com for further information)

3 Dose Mean = 25 kGy / Dose Min = 13 kGy / Dose Max = 37 kGy
(information obtained from dose mapping conducted on the diet product)

So,

5 kGy = 6 decimal place reduction
5/6 = 0.833 kGy = 1 decimal place reduction

Dose	Decimal place reduction	Expected Post Irradiated Bioburden
25 Kgy	25/0.833 = 30.0 decimal place reduction	$10*10^{-28}$ cfu/g
13 kGy	13/0.833 = 15.6 decimal place reduction	$2.5*10^{-14}$ cfu/g ^
37 kGy	37/0.833 = 44.4 decimal place reduction	$4.0*10^{-43}$ cfu/g

^To calculate: $2.5*10^{-14}$ cfu/g = $1/(10^{15.6 \text{ Decimal place reduction}})*100$ Initial Bioburden cfu/g
NOTE: All expected results are below the LOD for Salmonella spp.

EXAMPLE 2

Low sensitivity high bioburden normal dose
(Highly unlikely scenario)

Expected post irradiated bioburden using dose, published sensitivity figures and initial bioburden.

Known information

1 Initial bioburden of micro organism i.e. Bacillus pumilus of 50,000 cfu/g *(this is a spore forming bacteria which is relatively not very sensitive to gamma irradiation. It is highly unlikely to be found in a research diet but makes a good 'Low sensitivity' example)*

2 B. pumilus dose sensitivity of <30.0 kGy for a 10^6 (6 decimal place) reduction in numbers. *(See www.isotron.com for further information)*

3 Dose Mean = 25 kGy / Dose Min = 13 kGy / Dose Max = 37 kGy *(information obtained from dose mapping conducted on the diet product)*

So,

30 kGy = 6 decimal place reduction
30/6 = 5 kGy = 1 decimal place reduction

Dose	Decimal place reduction	Expected Post Irradiated Bioburden
25 Kgy	25/5 = 5.0 decimal place reduction	0.5 cfu/g
13 kGy	13/5 = 2.6 decimal place reduction	126 cfu/g ^
37 kGy	37/5 = 7.4 decimal place reduction	0.00199 cfu/g^

^To calculate: 126 cfu/g = $1/(10^{2.6 \text{ Decimal place reduction}})$*50,000 Initial Bioburden cfu/g
NOTE: Dose minimum will result in some detectable levels but it is unlikely that the initial bioburden would be as high as 50,000 cfu/g in the first place.

EXAMPLE 3

Low sensitivity high bioburden normal/low dose
(Highly unlikely scenario)

Expected post irradiated bioburden using dose, published sensitivity figures and initial bioburden.

Known information

1 Initial bioburden of micro organism i.e. Clostridium spp. of 1,000 cfu/g *(this is a spore forming bacteria which is relatively not very sensitive to gamma irradiation and is highly unlikely to be found in a research diet but makes a good 'normal sensitivity' example)*

2 Clostridium spp. dose sensitivity of <30.0 kGy for a 10^6 (6 decimal place) reduction in numbers. *(See www.isotron.com for further information)*

3 Dose Mean = 10 kGy / Dose Min = 5.4 kGy / Dose Max = 14.6 kGy *(information obtained from dose mapping conducted on a diet product)*

So,

30 kGy = 6 decimal place reduction
30/6 = 5 kGy = 1 decimal place reduction

Dose	Decimal place reduction	Expected Post Irradiated Bioburden
10 Kgy	10/5 = 2.00 decimal place reduction	10.0 cfu/g
5.4 kGy	5.4/5 = 1.08 decimal place reduction	83.2 cfu/g ^
14.6 kGy	14.6/5 = 2.92 decimal place reduction	1.2 cfu/g

^To calculate: 83.2 cfu/g = $1/(10^{1.08 \text{ Decimal place reduction}})$*1000 Initial Bioburden cfu/g
NOTE: Dose minimum will result in some detectable levels but it is unlikely that the initial bioburden would be as high as 50,000 cfu/g in the first place.