

APPLICATIONS OF IONIZING RADIATION: IRRADIATION OF BIOLOGICAL MATERIALS AND SMALL ANIMALS IN RESEARCH

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Abstract

Events induced by ionizing radiation are used in diverse fields of scientific research, and the application of this technology has for many years offered advantages when compared with alternative approaches.

Today, biological irradiators are established as one of the basic tools in diverse areas of biological research. Most Spanish biological research institutes are equipped with X-ray or gamma ray irradiators, which are used for many different research purposes. The risk associated with the use of this equipment is negligible, since radiation sources are effectively shielded and the equipment is fitted with multiple safety mechanisms. These negligible risks are verified by dosimetry reports for operators and researchers. In addition, in Category I gamma biological irradiators with a self-shielded source, the gamma emitter cannot be accessed by operators, increasing operational safety. This type of gamma irradiator can be used for irradiation of experimental animals and various kinds of tissue sample, and is a standard methodology for biological research in fields such as cardiovascular research and cancer.

Considering these points, the aim of this paper is to review the use of industrial sources without radiological risk in cell and animal irradiators for biological research. The paper describes the technical characteristics of equipment, facility design and the techniques and applications of irradiation of biological samples used in research.

KEY WORDS: *irradiator, biomedical research, self-shielded source.*

1. INTRODUCTION

Most Spanish biological research institutes are equipped with X-ray or gamma ray irradiators, which are used for a large number of research purposes.

The publication of IAEA Safety Series 102 set out, in summary form, the use of radioactive sources in industry, medicine, research and teaching. Other publications of the Agency provide more specific descriptions of the industrial applications of research irradiators.

The aim of this paper is to review the safe use of industrial sources in cell and animal irradiators for biological research, and describes technical characteristics of these equipments and facility design features according to national and international regulations^[1,2,3].

2. IRRADIATION IN RESEARCH

Today, biological irradiators are established as one of the basic tools in diverse areas of biological research. Biological gamma irradiators are used extensively for irradiation of experimental animals and cells in suspension or adherent culture.

The different categories of gamma irradiators are the following:

- Category I: The encapsulated source is loaded into a container constructed of solid materials, and it is not possible for users to access the source and irradiation chamber during irradiation.
- Category II: Panoramic gamma irradiators, in which the source is loaded into a container constructed of solid materials when in storage position; access to the irradiation room is not permitted when the source is not in storage position.
- Category III: Irradiators with an encapsulated gamma source permanently submerged in a pool of water, making it physically impossible for staff to access the source and irradiation chamber.

- Category IV: Panoramic gamma irradiators in which the encapsulated source is immersed in a pool of water when not in use; access to the irradiation room is not permitted when the irradiation source is not in storage position.

2.1. Technical specifications of biological irradiators

The gamma irradiators used in biological research are category I autonomous irradiators with dry storage. They are designed to cover the full range of applications in biological research irradiation (figure 1a and b).



Fig.1a. A biological gamma irradiator



Fig.1b. Interior of a biological gamma irradiator

This equipment allows irradiation of test tubes, culture dishes, petri dishes, and cannisters that occupy the entire irradiator chamber to irradiate several mice, rats, etc. Samples and animals can be exposed to dose rates ranging from 1 to 100% of the maximum available dose rate. Different models differ in the size of the chamber and the activity of loaded gamma radiation sources.

The irradiation chamber is accessed through a shielded door which is the same size as the inner chamber and fitted with safety locks. The chamber also has access tubes for introducing gases or specific sensors if required by the experiment. Selected areas of several animals can be irradiated simultaneously with the use of a collimator system.

The irradiation chamber is isolated from the operation system of the source, so that spills or corrosive materials generated or placed in the irradiation chamber can be removed without affecting the operation mechanism.

Most irradiators use Cesium-137^[3] as radioactive material because of its long half-life (30 years), which eliminates the need for frequent dosimetric corrections and costly refills. Moreover, the emission energy of Cs-137 (662KeV) is less than that of the alternative emitter Cobalt-60, and therefore requires less shielding. The most commonly used Cs-137 activities are between 18.5 and 111 TBq (500 and 3000 Ci), although the equipment can be loaded with up to 444 TBq (12000 Ci) if necessary.

The usual doses used are 2-25 Gy (200-2500 rads) for experimental animals and 5-50 Gy (500-5000 rads) for cell cultures. Doses and exposure times for live animals and cells should be minimized, justifying the source activities indicated above; however, a wide range of Cesium-137 sources is available to cover other dose requirements.

The positioning of the source in the source holder produces optimal isodose curves in the chamber. The source holder is placed in a duct at the back of the irradiation chamber that is shielded behind by the lead shielding during use, and completely shielded when stored.

Samples rotate in front of the source holder duct in one of three positions, thus producing different dose rates according to the distance from the source.

2.2. Facility design

Gamma irradiators are high energy photon emitters. These devices have a very low risk of contamination due to the double encapsulation of the radioactive source and their certification as Special Form. The risk of radiation can also be considered negligible due to the multiple security systems and specific shielding used.

The dose rate at the outer surface of the apparatus does not exceed $30\mu\text{Sv/h}$. Nonetheless, due to the total activity of the cesium-137 source, the irradiator facility is classified as a controlled area with radiation risk.



Fig.2. Irradiator facility sign

Corresponding sign for the area is a green radiation warning symbol with radial spikes indicating radiation risk. The sign also carries text above the symbol indicating the type of area and below the symbol indicating the nature of the hazard (figure 2).

Thanks to the shielding of the equipment, the irradiator room does not require additional shielding. Nonetheless, since the facility is used for the manipulation of biological material all surfaces are selected to allow easy decontamination. The floor of the room is reinforced to support the weight of the irradiator.

Radiation levels are monitored with a rate alarm meter linked to sound and bright-light alarm indicators. The monitor is connected electronically to the equipment and automatically stops exposure (by retracting the source holder to the safe position) if a high dose rate is detected during irradiation. Access to the irradiator room is moreover restricted to operators and authorized personnel. Current regulations are followed regarding irradiator room design and periodic checks of dose rate detectors, warning signs, alarms, and fire-fighting systems.

2.3. Functions of personnel and dosimetry.

The following personnel take charge of the manipulation of the gamma irradiator (qualifications and licenses are those required under local regulations by the competent Spanish authorities):

- Personnel in charge of the manipulation of the gamma irradiator: a team of assigned technicians, all holding an operator license in the application field and working under the supervision of the head of the radioactive facility (RSO, Supervisor or Head of Radiation Protection).
- Research Personnel: carrying out research procedures and stating the dose to be applied to their samples.

Generally, all exposed personnel are defined as exposed workers in category B (according to Spanish legislation), since it is improbable that they will receive doses above 6 mSv (3/10 of the limit for Category A) in a calendar year. Dosimetric monitoring of occupationally exposed Category B personnel is controlled by means of personal dosimeters. In research centres, thermoluminescence dosimeters are used, and the whole body dose is monitored by means of a lapel TLD dosimeter worn throughout the working day.

Since the introduction of these systems, doses received by exposed workers operating the equipment during irradiation work have not even reached the legal limits for members of the general public (1 mSv/year). It can therefore be concluded that work with high activity sources as used in this type of equipment **does not carry a significant risk of radiation exposure**.

3. TECHNIQUES AND APPLICATIONS

For routine irradiation of animals and samples the irradiator is operated from the control console, eliminating the risk associated with source handling. These operations are performed by Radiation Protection Service personnel who hold an operator or supervisor license.

The material or animal to be irradiated is placed in the irradiator chamber in front of the radioactive source, such that the energy absorbed (dose) will depend on the activity of the source, the distance from the source, and the exposure time.

This is a simple and safe technology. The distance from the sample to the source (irradiation position 1, 2 or 3; figure 3) is chosen according to the isodose curves and the dose needed to obtain the desired effects; therefore the only parameter to bear in mind is the exposure time needed to reach the required dose. This simple control provides unique benefits in terms of repeatability and traceability.

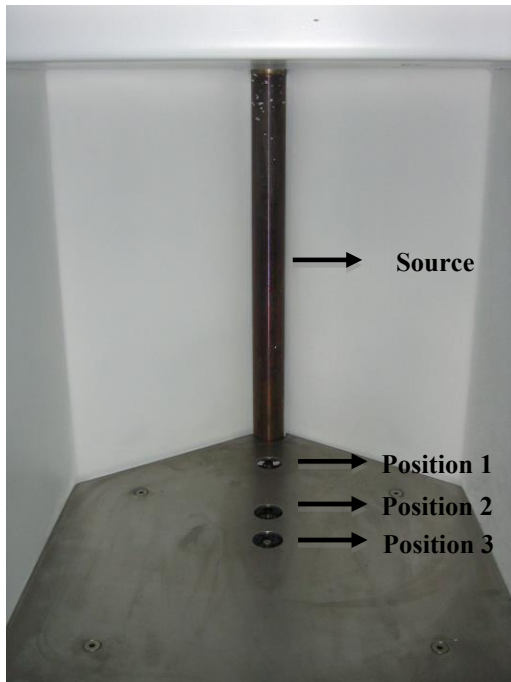


Fig.3. Positions of sample placement relative to the radioaction source.

The sample holder rotates during the irradiation at uniform speed to ensure a uniform distribution of the dose.

For better control of the samples, the gamma irradiator is equipped with a variety of accessories adapted to the needs of different irradiation samples (figure 4).



Fig. 4: Sample holders for mice (left and centre) and biological samples (right)

Irradiation is a fundamental methodology in applied research and finds application in:

- Generation of chimeric animals.
- Induction of immunodeficiency
- Retroviral gene transfer.
- Development of preclinical models of gene therapy.
- Implantation of non-isogenic hybridomas.
- Maintenance and differentiation of cell lines.
- Developing new cell lines.

- Locking the cell cycle and induction of apoptosis.
- Analysis of the susceptibility of cell lines to radiation.
- Elimination of endogenous hematopoiesis.

Below we describe examples of techniques and applications of the irradiation of biological material and small animals that are used in research centres.

3.1. Irradiation of biological materials.

Cells:

Many research procedures that require gamma irradiation are conducted with mouse fibroblasts^[5]. Mouse fibroblasts are generally exposed to a gamma irradiation dose of 10 Gy (figure 5), and the irradiation normally has one of two goals:

- Inactivation of cell proliferation: The radiation does not kill the fibroblasts but they can no longer divide. In this condition, these cells are used as the substrate ("feeders") on which stem cells are seeded in order to maintain them in the undifferentiated, pluripotent state.
- Production of conditioned medium: Irradiation of fibroblasts causes them to release a number of factors to the medium, which can be collected by the researcher for later analysis in other bioassays.

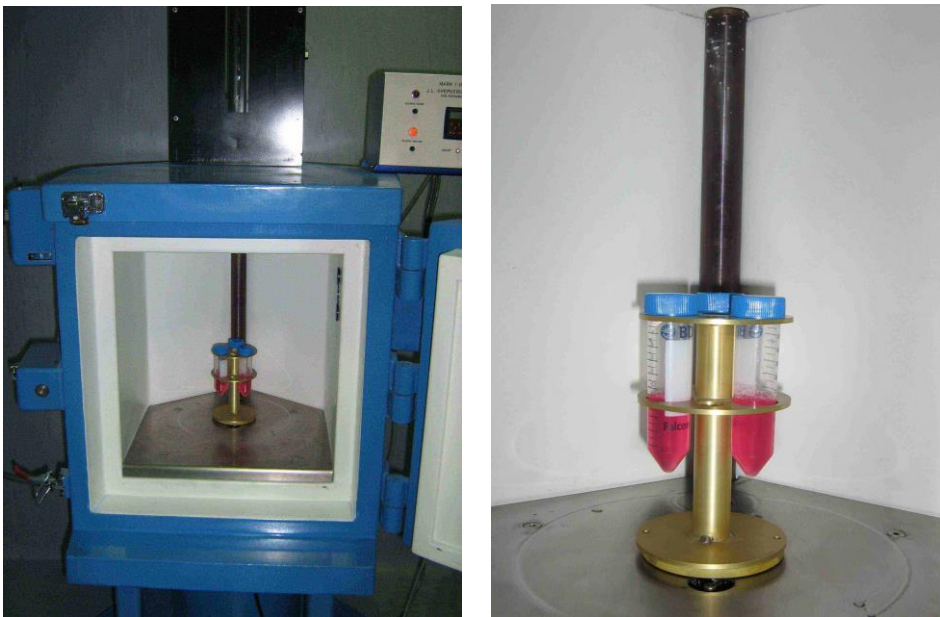


Fig. 5: Cell suspensions prepared for irradiation

3.2. Irradiation of small animals.

Mice:

A widely used application of irradiation in biomedical research is its use to render animals immunodeficient, thereby permitting the study of the reconstitution of the immune system by injecting bone marrow cells from non-irradiated donors^[6]. The generation of these bone marrow chimeric animals (which contain a mix of their own cells and bone marrow from the donor) is important for experimental studies of cellular and humoral immunity. The technique (figure 6) consists of applying a lethal dose (around 9.5 Gy) (figure 7a and 7b) that kills proliferating bone marrow cells, thus

eliminating the circulatory and immune system of the animal. On the same day another mouse is sacrificed and its bone marrow is transplanted into the irradiated mouse. The recipient mouse is a chimera. The aim of the procedure is to establish hematopoiesis (the formation, development and maturation of blood constituents: erythrocytes, leukocytes and platelets) driven by genetically marked cells in recipient animals.

After several weeks, the presence of donor-derived cells can be evaluated in the peripheral blood or primary and secondary lymphoid organs of transplant recipients. The animal is subsequently sacrificed and its organs, tissues and cells processed for further analysis.

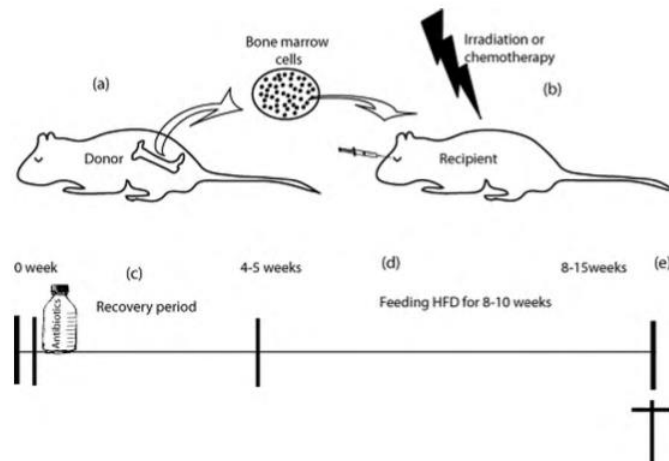


Fig. 6: Bone marrow transplantation procedure



Fig. 7a: Mice in a multi-holder ready for irradiation.



Fig. 7b: A mouse in an individual holder ready for irradiation.

Fish:

Another recent research application of irradiation is the study of tumour cell transplantation, especially important for testing of malignancy of these cells and their migration capacity^[7,8]. Transplantation studies are also essential for identifying cancer stem cell populations. This kind of study commonly uses the zebrafish due to its ease of observation (figure 8).

An important consideration in these tests is the need to prevent rejection of the graft. Therefore irradiation is often used to suppress the immune system of the recipient adult fish and thereby prevent rejection of the transplanted cells. The doses used, in the range of 20 to 25 Gy, are sublethal, and are tolerated by at least 90% of fish (figure 9a and b).

After irradiation, cells isolated directly from a tumour in another zebrafish are transplanted into immunosuppressed fish. Successful grafting is determined by observation of the post-transplant recipient animals. This is the fundamental limitation of all transplant studies, since a tumour transplanted into the peritoneal cavity may not be visible from the outside but can still cause the death of the recipient. With further development of imaging techniques and other tools, tumour transplantation in zebrafish continues to contribute to the understanding of the biology of tumour cells.

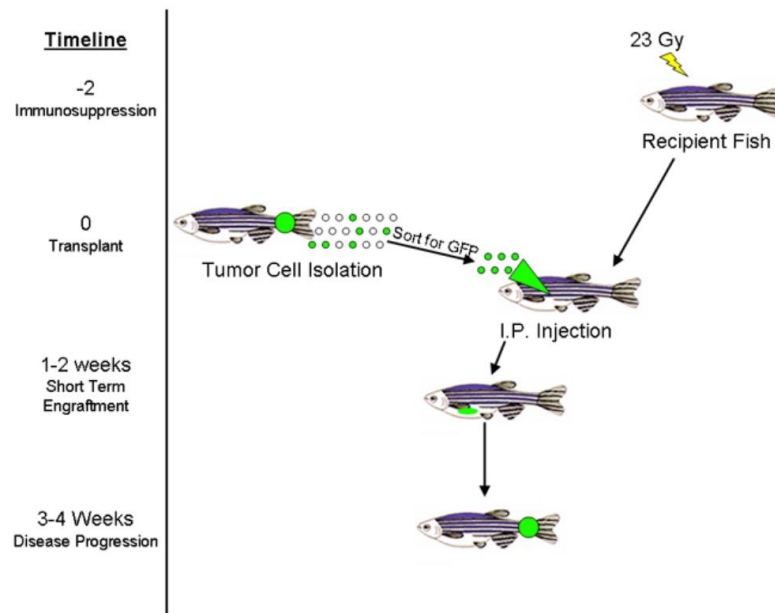


Fig. 8: Study of tumour cells in zebrafish



Fig. 9a: Zebrafish ready for irradiation.

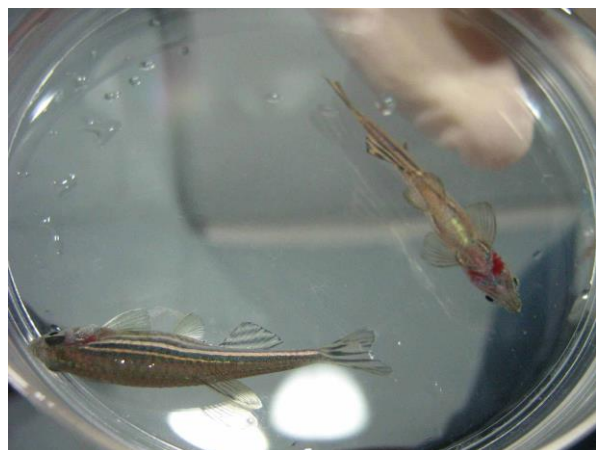


Fig. 9b: Zebrafish ready for irradiation.

4. CONCLUSIONS

Biological gamma irradiators are established as a basic tool in diverse areas of biological research. The risk associated with the use of high activity sources loaded into this type of equipment is negligible, since radiation sources are effectively shielded and the equipment is fitted with multiple safety mechanisms. These negligible risks are checked by operators and researchers dosimetry reports. The versatility, simple operation, unique safety features, and availability of many accessories make it practical for a research institute to house a single irradiator located in a central laboratory location,

even in clean areas, with minimal levels of external radiation in accordance with the ALARA philosophy.

5. REFERENCES

- [1] Royal Decree 1836/1999, of December 3, by which the Regulation on nuclear and radioactive facilities is approved. (modified by RD 35/2008)
- [2] Royal Decree 783/2001, of July 6, by which the Regulation on sanitary protection against ionizing radiation is approved. (modified by RD 439/2010)
- [3] Sánchez, A. et al. Manual de Protección Radiológica en Centros de Investigación Biológica. Sociedad Española de Protección Radiológica. SEPR nº 3. Madrid. (2001)
- [4] J. L. Shepherd & Associates. Manual of self-contained category I Cs-137 irradiators for biological and other low research, Mark I Series.
- [5] David A. Conner. Current Protocols in Molecular Biology (2000) 23.2.1-23.2.7
- [6] Aparicio-Vergara M, et al. Bone marrow transplantation in mice as a tool for studying the role of hematopoietic cells in metabolic and cardiovascular diseases. *Atherosclerosis* (2010), doi:10.1016/j.atherosclerosis.2010.05.030
- [7] Traver D, Winzeler A, Stern HM, Mayhall EA, Langenau DM, Kutok JL, et al. Effects of lethal irradiation in zebrafish and rescue by hematopoietic cell transplantation. *Blood* 2004;104:1298–1305. [PubMed: 15142873]
- [8] Zebrafish. 2009 December ; 6(4): 339–346. doi:10.1089/zeb.2009.0607.