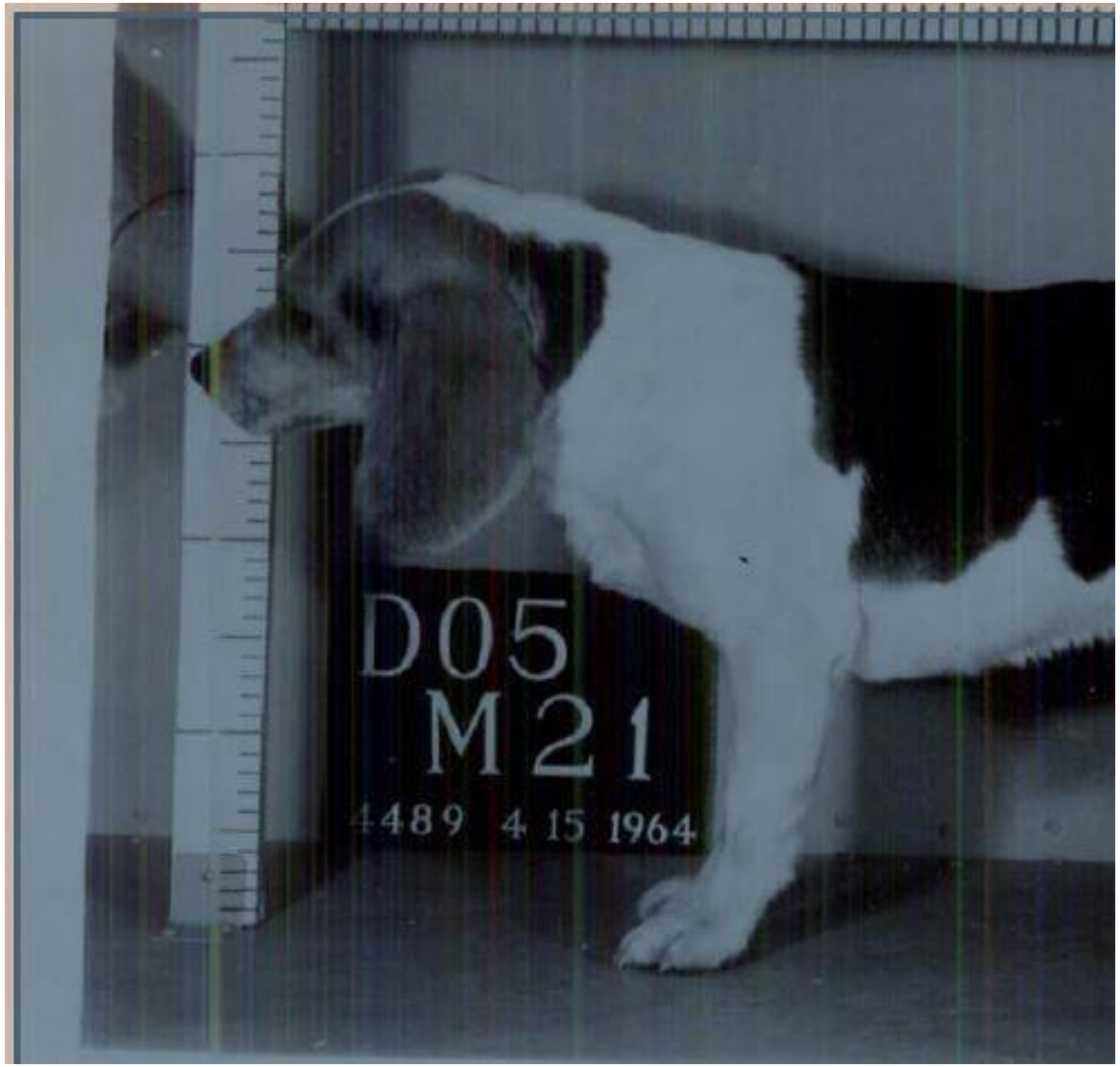
Animal Studies

Dr. Gayle Woloschak



Current Challenges within existing models

High LET radiation does not generally have a DDREF or DREF associated with it; so studies estimating DDREF and DREF have generally naturally focused on low LET studies

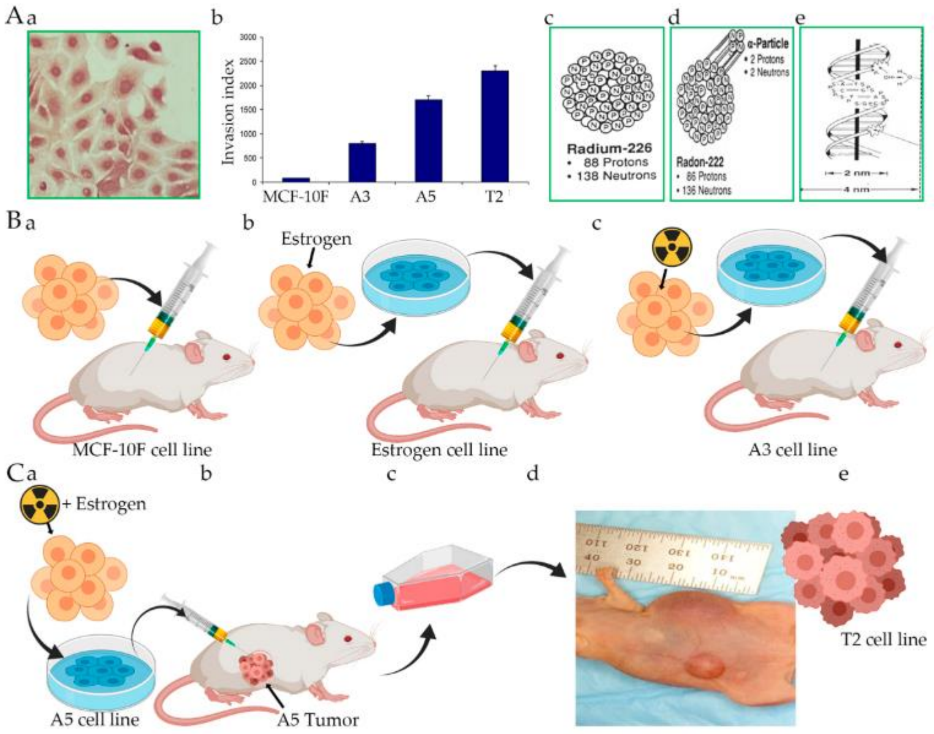
The existing archives of irradiated animal studies contain information about historical animal experiments concluded by the 2000's, and include samples from hundreds of thousands of animals exposed to different qualities and doses/dose rates of radiation.

Efforts to use experimental animal data for DDREF estimates were a subject of renewed interest in recent years (Haley et al., 2015; Hoel, 2015)

Absence of accessible digital versions of the available data for some of the older as well as more recent animal studies.

Recent Data after 2000’s

Some recent low-dose radiation data were obtained with transgenic animals with genetic susceptibilities for accumulation of mutations or other endpoint(s) under consideration.



Calaf GM, Crispin LA, Roy D, Aguayo F, Muñoz JP, Bleak TC. Gene Signatures Induced by Ionizing Radiation as Prognostic Tools in an In Vitro Experimental Breast Cancer Model. *Cancers*. 2021; 13(18):4571. https://doi.org/10.3390/cancers13184571

Recent Data after 2000's

Additional low-dose studies to consider:

- **Bakshi et al., 2016;**
- **Hofig et al., 2016.**
- **Mancuso et al., 2015**

Mancuso et al (2015), proved that mice acutely exposed to low doses of 0.3 or 6 Gy showed increased atherogenesis compared to age-matched controls, and this effect was persistent.

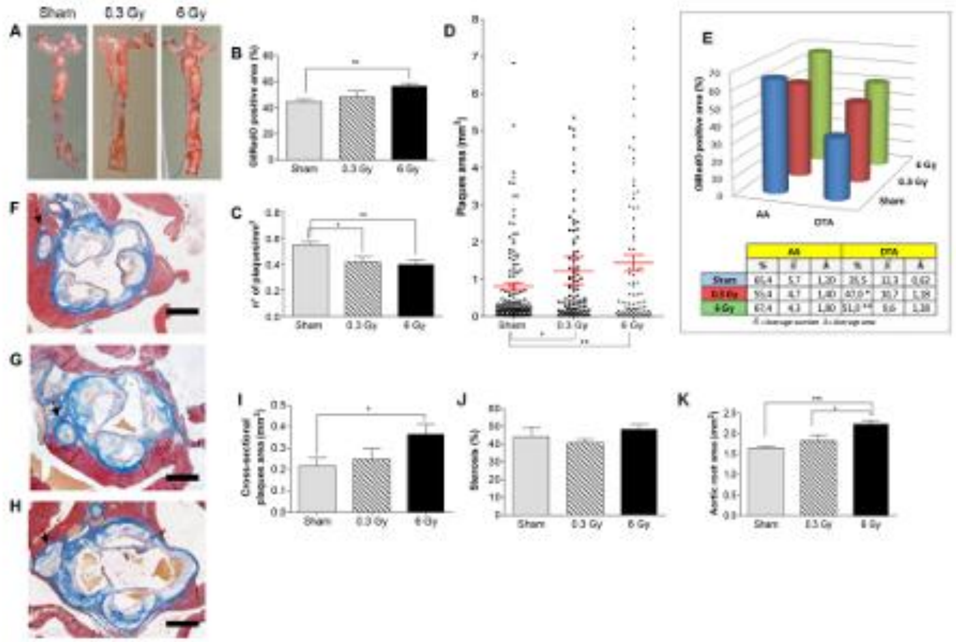


Figure 3: Chronic irradiation. A. Representative ORO-stained aortas from female *ApoE*$^{-/-}$ mice after chronic irradiation with 0.3 Gy or 6 Gy over 300 days, and age-matched controls. Graphic representation of quantitative analyses performed on digital images from en face preparations of aortas ($n = 8$) showing: **B.** Percentage of ORO-stained aortic area. **C.** Plaque density. **D.** Plaques size. **E.** Regional distribution. **F–H.** Masson's trichrome staining of aortic root cross-sections from each experimental group. **I–K.** Dimensional analyses of plaques area (I), aortic stenosis (J) and aortic total area (K). Data are shown as mean ± SEM. Differences were tested with Student's *t*-test. $^{*}P < 0.05$; $^{**}P < 0.001$; $^{***}P < 0.0001$. Arrows: coronary artery. Bars: 500 μm.

Mancuso, Mariateresa, Emanuela Pasquali, Ignacia Braga-Tanaka, Satoshi Tanaka, Alessandro Pannicelli, Paola Giardullo, Simonetta Pazzaglia, Soile Tapio, Michael J Atkinson, and Anna Saran. "Acceleration of Atherogenesis in ApoE−/− Mice Exposed to Acute or Low-Dose-Rate Ionizing Radiation." *Oncotarget* 6, no. 31 (2015): 31263–71.

Efforts to Improve the DDREF Estimate and the Calculations of LDEF and DREF Focus on:

Development of New Computational Models:

- Future studies may range from detailed, experiment-specific investigations, and meta-analyses focusing on parameters of dosimetry and specific animal and strain characteristics.
- Data could be used to supplement and support information from the epidemiology.
- Comparisons of these mouse and rat studies have shown that there are strain-specific differences and age-specific differences (with the young being more radiosensitive) for dose- and dose-rate-responses that need to be considered.

Collection of Additional Data:

- Detailed data archiving is an important step in arriving at DDREF estimates.
- More effort needed to archive all of the existing data on irradiated animals or extend archival efforts to new animal studies.

Historic Studies on Irradiated Animals Suitable for DDREF, DREF and LDEF Evaluation

european radiobiological archives

Studies encompassed in ERA include rodents of different families, species and strains as well as dogs and a few other species

Northwestern University Radiobiology Archives

Most of the studies in the Northwestern Janus Archive, that is partially recombined with the National Radiobiology Archives, are aimed to understand the effects of external beam neutron and Cobalt-60 gamma-ray irradiation on lifespan and tumourigenesis across a wide range of dose and dose-rate patterns. In addition, beagle dogs were also used for inhalation and injection experiments in the US; the radionuclides used included ^{241}Am, ^{144}Ce, $^{249, 252}$Cf, ^{253}Es, ^{237}Np, $^{237, 238, 239}$Pu, $^{226, 228}$Ra, ^{90}Sr, ^{228}Th, and $^{90, 91}$Y; these were delivered via inhalation or injection, in different chemical forms modulating bioavailability and biokinetics (Puukila et al., 2017, 2018).

Historic Studies on Irradiated Animals Suitable for DDREF, DREF and LDEF Evaluation

NASA GeneLab holds only datasets (no archives tissues)

More than 23,000 animals involving mice, rats, rabbits, dogs, pigs and monkeys - *Undigitized*

Canadian Nuclear Laboratories

CNL is creating new efforts to develop datasets and tissue archives from animals exposed to radiation that would be available to the public

量子科学技術研究開発機構
QST National Institutes for Quantum and Radiological Science and Technology

The institution of environmental sciences

Evaluating effects of radiation and risk analyses for life shortening and cancer prevalence using laboratory animals.

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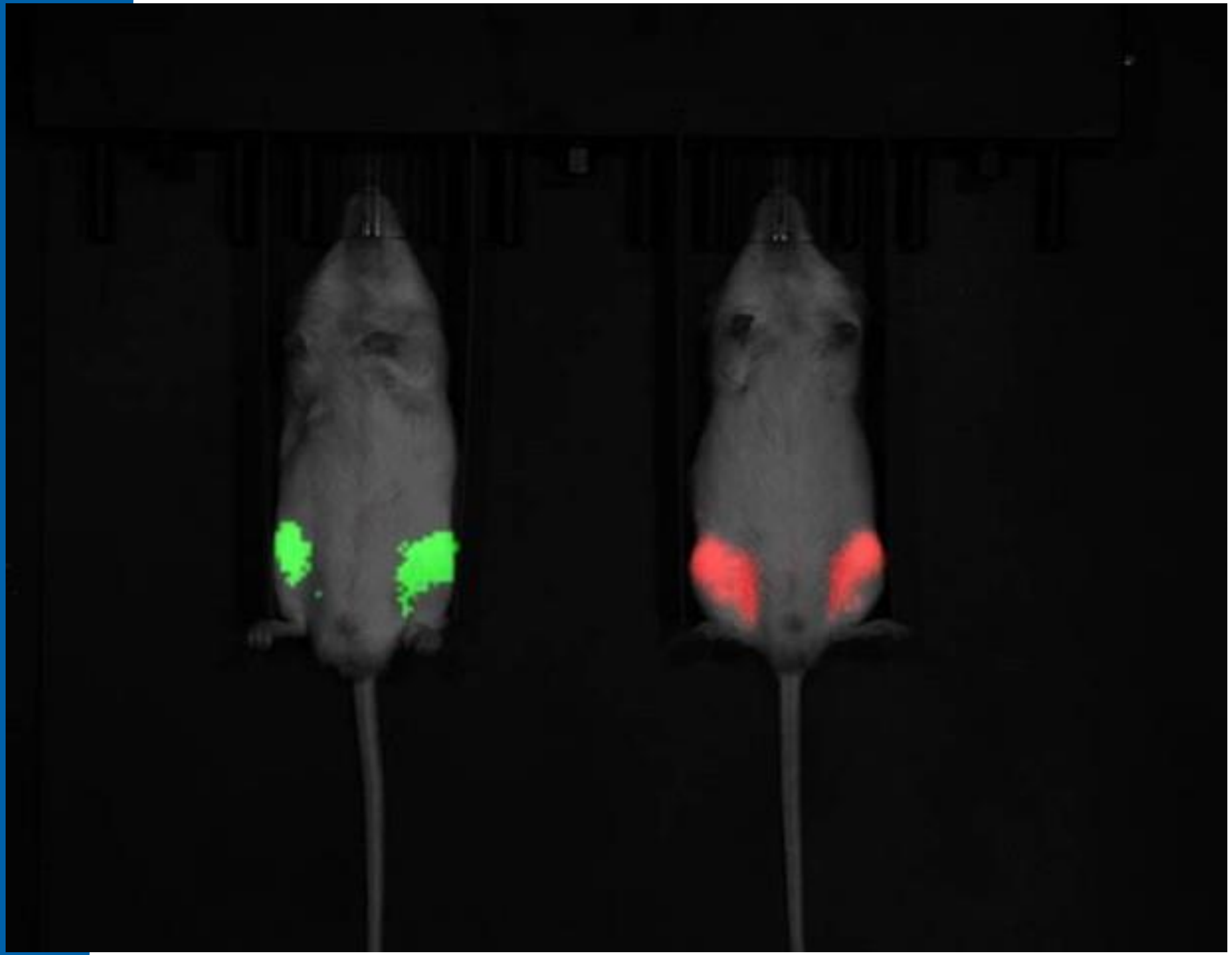
DDREF Study Table section

Irradiated animals exposed to low dose & low dose rate



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DDREF Studies

Studies on Irradiated Animals after 2000's Suitable for DREF and LDEF Evaluation

Dynamics of radiation exposure	Source	Animals, strain, age etc.	Number of animals	Doses and/or Dose Rates	Study Endpoint	Reference
Environmental contamination exposures	Fukushima	Domestic and 'wild'	302 cattle, 57 pigs, 200 macaque, 8 wild pigs, 5 horses	NA	NA	Takahashi et al., 2015
Environmental contamination exposures	Fukushima		9	NA	NA	Takino et al., 2017
Experimental internal exposure	^{137}Cs injection	Mice	NA		Spermatogenesis process alterations caused by radiation	Nakajima et al., 2015
Experimental chronic external beam	^{137}Cs-gamma ray	Mice, C3H/HeN 56 days and older	NA	1, 20, 400 or 890 mGy per day	chromosomal aberrations in splenic lymphocytes noticeable above 400 mGy per day	Tanaka et al., 2013
Experimental chronic external beam	^{137}Cs-gamma ray	Mice, C3H/HeN 56 days and older	215 (Tanaka et al., 2008) / 468 (Tanaka et al., 2009)	1, 20, 400 or 890 mGy per day	Linear increase of aberrations below 3 Gy for dose rate 400 mGy per day and below 8 Gy for exposures of 20mGy day^{-1}	Tanaka et al., 2008, Tanaka et al., 2009
Experimental chronic external beam	^{137}Cs-gamma ray	Mice B6C3F1	1129	20 mGy day^{-1} for 400 days	Obesity and fatty degeneration of the liver and different degenerative changes in adrenal glands and ovaries	Tanaka et al., 2017
Experimental chronic external beam	^{137}Cs-gamma ray	Mice, males only B6C3F1/Jcl	250	20 mGy day^{-1} for 400 days	A calorie restriction diet extends lifespan of irradiated mice and controls	Yamauchi et al., 2019
Experimental chronic external beam	^{137}Cs-gamma ray	Mice: B6C3F1, C3H, C57Bl/6, (gpt delta × SWR) F1 mice	4000	Total doses 20–21, 400–420 or 8,000–8,050 mGy delivered over 400-485 days	Review of IES findings focused on lifespan, neoplasm incidence, body weight, tumour cell transplantability, changes in chromosome structure, gene mutations, changes in mRNA and protein levels, and transgenerational effects on lifespan and neoplasm incidence	Braga-Tanaka et al., 2018

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Continued - DDREF Studies

Irradiated animals exposed to low dose & low dose rate

Dynamics of radiation exposure	Source	Animals, strain, age etc.	Number of animals	Doses and/or Dose Rates	Study Endpoint	Reference
Experimental external beam	Variety: fast neutrons gamma ray	Rodents: Mice and Rats	NA (review)	Variety of conditions	Review paper listing 9 mGy of fast neutrons or 100 mGy of gamma rays are required for detectable changes in comet assay or micronuclei frequency	Shimura and Kojima, 2018
Experimental external beam	^{137}Cs-gamma ray	B6C3F1 mice, 35 to 365 days of age at the beginning of irradiation	4000	Acute vs. chronic exposures	The age of exposure influenced sensitivity to cancer with a weight ratio greater than five. Chronic exposure to 21 mGy day^{-1} had 0.33 fold risk of malignant cancer development compared to acute exposure.	Doi et al., 2020
Experimental fractionated, external beam	X-ray and gamma ray	Mice, C57BL/6	72	7.2 Gy in 1.8 Gy fractions, w or w/o low-dose treatment	Incidence of lymphoma reduced with 75 mGy X-ray pre-treatment 6h before fractions or continuous exposure to gamma rays at 1.2 mGy h^{-1}	Ina et al., 2005
Experimental external beam	Gamma ray	Mice	615	1.4 mGy h^{-1} for 45 days	DNA damage	Graupner et al., 2016
Experimental fractionated, external beam	X-ray	Mice C57BL/6 ‘resistant’ and BALB/c ‘sensitive’	54	four weekly doses of 75 mGy or 1.8 Gy per week	Breast cancer development and gene expression; gene expression profiles strain and dose specific	Snijders et al., 2012
Experimental external beam	X-ray	Mice	NA	1 Gy and 100 mGy exposures	Evaluation of T cell receptor spectra: 100 mGy exposure (but not 1Gy) caused accelerated aging as shown by loss of receptor diversity	Candeias et al., 2017

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Continued - DDREF Studies

Irradiated animals exposed to low dose & low dose rate

Dynamics of radiation exposure	Source	Animals, strain, age etc.	Number of animals	Doses and/or Dose Rates	Study Endpoint	Reference
Experimental external beam	Gamma ray	Wild-type C57BL/6 mice and their p53+/- counterparts	1824	5 days a week exposures to 48, 97 or 146 mGy total at dose rate 0.7 mGy h^{-1}	Life shortening and increased cancer incidence in wild type mice exposed for 30 or 60 weeks, but not 90 weeks. No differences in heterozygotes	Mitchel et al., 2008
Experimental external beam	X-ray	Wild-type C57BL/6 mice and their p53+/- counterparts	474	10 mGy	A single 10 mGy x-ray exposure significantly delayed onset of cancer in irradiated compared to unirradiated p53 +/- animals	Lemon et al., 2017
Experimental external beam	X-ray	Mice, inducible expression of the Ki-rasG12C gene in lungs	NA	80 -160 mGy	Irradiation increases cancer incidence, especially in female mice	Munley et al., 2011
Experimental external beam		Mice, Ptch1+/-	NA	50 mGy	DNA damage, death of sebaceous gland cells but reprogramming of bulge epidermal stem cells and increase in skin cancer over baseline	Revenco et al., 2017
Experimental external beam	Gamma ray	Mice, Ptch1+/-	NA	100-500 mGy total delivered at 25 - 125 mGy day^{-1}	100 mGy dose mutation pattern and spontaneous mutation patters identical while 500 mGy caused LOH that was different; studies included radiation-induce medulloblastoma	Tsuruoka et al., 2016
Experimental external beam	Gamma ray	PU.1 inactivated mouse	50	3 Gy given at 20 mGy day^{-1}, 200 mGy day^{-1}, or 1000 mGy day^{-1}	Development of AML in mice is dose-rate dependent in hematopoietic stem cells	Ojima et al., 2019

Continued - DDREF Studies

Irradiated animals exposed to low dose & low dose rate

Dynamics of radiation exposure	Source	Animals, strain, age etc.	Number of animals	Doses and/or Dose Rates	Study Endpoint	Reference
External beam	Gamma ray	Mice	NA	4 or 8 Gy at 20 mGy day^{-1}	Different protein expression at low-dose-rate and high-dose-rate exposures	Nakajima et al., 2017
External beam	Gamma-ray	Rat	531	0.5-8 Gy total dose delivered at 3-30 Gy h^{-1}	Induction of mammary carcinoma with continuous exposure was age dependent	Imaoka et al., 2019
External beam	Gamma-rays and X-rays	Mice, rats	11,528	Range of doses under 4 Gy	Life shortening of 11,528 rodents of mixed gender comparing doses under 4Gy; DREF of 2 was reported; comparing doses under 3Gy gave DREF of 2.6 with larger error bars.	Haley et al., 2022

Major findings on DREF and LDEF found in this review from animal data sets.

Study	Endpoint	DDREF or DREF or LDEF or EER	Comments
Tran and Little, 2017 (gamma ray data only)	All tumours (lethal, coincident, non-lethal)	DREF = 1.190 (95% CI 0.861 to 1.723) LDEF = 1.056 (95% CI 0.992 to 1.139)	All doses (up to 49 Gy for fractionated exposures) projected to 1.5 Gy mathematically
Doi et al., 2020	All lethal tumours	DREF=3.0 (95% CI: 1.8, 5.1); If no age at exposure effects are considered, DREF=5.7 (95% CI: 4.0, 8.0)	Chronically (up to 8 Gy) vs acutely (up to 5.7 Gy) irradiated B6C3F1 mice
BEIR VII (Table 10-2)	Life shortening	'LSS' DDREF 0.1-3.2	Radiobiology animal experiments (total dose up to 1.5 Gy)
Hoel, 2015	Life shortening	DDREF at 1 Gy 2.3 RFM mice 2.4 BALB/c mice	Acute vs. chronic animal IR datasets from Storer et al 1997 (total dose up to 1 Gy)
Haley et al., 2015	Life shortening	'LSS' DDREF based on BEIR VII approach 2.9-infinity	All mice from ERA database (total dose up to 1.5 Gy)
		'LSS' DDREF based on BEIR VII approach 0.9-3	Only acute exposures from ERA database (total dose up to 1.5 Gy)
		'LSS' DDREF based on BEIR VII approach 4.8-infinity	Comparison acute vs. chronic ERA datasets on B6CF1, C57BL/Cnb, BALBc/Cnb (total dose up to 1.5 Gy)
Haley et al., 2022	Life shortening	DREF=2.0 for exposures of 0.25-4 Gy, 2.6 for exposures of 0.25-3 Gy	Acute and protracted exposures were compared for a variety of mouse and rat strains; no sex differences were observed.

Future Directions

- Combining studies of animals to acquire large datasets is especially important when examining low-dose and low-dose-rate effects where the number of animals affected by radiation may be small.

- Variations in low-dose and low-dose-rate responses to cancer induction in rodent systems suggest that calculations of LDEF and DREF may vary with each different cancer type. Animal studies have also suggested that many factors may influence low-dose radiation responses including genetic background, diet, overall health of the animal, and others.

- Additional animal models are needed for low-dose radiation research, although doses may be significantly different from one species to another.

- Addition of new rodent transgenic models should be considered

- A successful compilation would have to include both the existing archival data and the materials coming from new studies from NASA, Japanese chronic animal exposure studies, and others (Woloschak, 2016).

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