Science of the Total Environment 902 (2023) 166030

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

The challenges of defining hormesis in epidemiological studies: The case of radiation hormesis

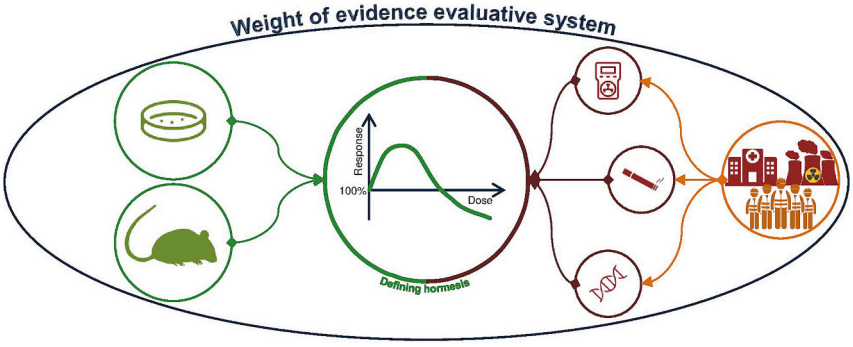
Ivo Iavicoli [a,*], Luca Fontana [a], Carolina Santocono [a], Davide Guarino [a], Martina Laudiero [a], Edward J. Calabrese [b]

[a] Department of Public Health, Section of Occupational Medicine, University of Naples Federico II, Via S. Pansini 5, 80131 Naples, Italy
[b] Department of Environmental Health Sciences, Morrill I, N344, University of Massachusetts, Amherst, MA 01003, USA

HIGHLIGHTS

- Ionizing radiation risk assessment should include the hormesis concept.
- Definition of hormesis in epidemiological studies is a very complex task.
- Lack of exposure doses or control groups hinder the possibility to detect hormesis.
- Epidemiological data should be integrated in a weight of evidence evaluative system.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Paola Verlicchi

Keywords:
Radiation hormesis
Ionizing radiation
LNT model
Risk assessment
Healthcare workers
Nuclear workers

ABSTRACT

In the current radiation protection system, preventive measures and occupational exposure limits for controlling occupational exposure to ionizing radiation are based on the linear no-threshold extrapolation model. However, currently an increasing body of evidence indicates that this paradigm predicts very poorly biological responses in the low-dose exposure region. In addition, several *in vitro* and *in vivo* studies demonstrated the presence of hormetic dose response curves correlated to ionizing radiation low exposure. In this regard, it is noteworthy that also the findings of different epidemiological studies, conducted in different categories of occupationally exposed workers (e.g., healthcare, nuclear industrial and aircrew workers), observed lower rates of mortality and/or morbidity from cancer and/or other diseases in exposed workers than in unexposed ones or in the general population, then suggesting the possible occurrence of hormesis. Nevertheless, these results should be considered with caution since the identification of hormetic response in epidemiological studies is rather challenging because of a number of major limitations. In this regard, some of the most remarkable shortcomings found in epidemiological studies performed in workers exposed to ionizing radiation are represented by lack or inadequate definition of exposure doses, use of surrogates of exposure, narrow dose ranges, lack of proper control groups and poor evaluation of confounding factors. Therefore, considering the valuable role and contribution that epidemiological studies might provide to the complex risk assessment and management process, there is a clear and urgent need to overcome the aforementioned limits in order to achieve an adequate, useful and more

* Corresponding author at: Department of Public Health, Section of Occupational Medicine, University of Naples Federico II, Via S. Pansini 5, 80131 Naples, Italy.
E-mail address: ivo.iavicoli@unina.it (I. Iavicoli).

https://doi.org/10.1016/j.scitotenv.2023.166030
Received 16 May 2023; Received in revised form 24 July 2023; Accepted 1 August 2023
Available online 5 August 2023

real-life risk assessment that should also include the key concept of hormesis. Thus, in the present conceptual article we also discuss and provide possible approaches to improve the capacity of epidemiological studies to identify/define the hormetic response and consequently improve the complex process of risk assessment of ionizing radiation at low exposure doses.

1. Introduction

Hormesis is a biphasic dose/concentration response, in which low doses/concentrations induce stimulation, and high doses/concentrations induce inhibition (Calabrese, 2008; Calabrese and Baldwin, 2002; Calabrese and Blain, 2005, 2011; Calabrese and Mattson, 2011; Mattson, 2008). The quantitative features of hormesis are characterized by a maximum stimulatory response, usually between 30 % to 60 % greater than the control group, with a stimulatory zone width that is typically in a 10–20-fold range starting immediately below the toxicological/pharmacological threshold. However, the stimulatory zone width often displays widespread variability, and frequently exceeds more than 50-fold. The hormetic response may be induced by a direct sub-toxic (hormetic) dose, and/or sub-toxic (hormetic) preconditioning dose, which is followed by a toxic dose (Calabrese, 2016a, 2016b), or an overcompensation to a disruption in homeostasis (Calabrese, 1999, 2008). Hormesis is a broadly generalizable dose response phenomenon, whose occurrence is independent of biological model, inducing agent, endpoint and mechanism (Calabrese, 2013; Calabrese and Kozumbo, 2021) and it has a greater than century-long historical foundation within the chemical and radiation biology (Calabrese and Baldwin, 2000a, 2000b, 2000c, 2000d, 2000e). The term hormesis was first used in the scientific literature in 1943 by Southam and Ehrlich in studies on the effects of extracts of the red cedar tree on the growth of multiple fungal species. However, the concept of a biphasic dose concentration response long preceded the report of Southam and Ehrlich (1943) with the first substantial experimental reporting of this phenomenon given by Hugo Schultz, a professor at the University of Grieswald at a local medical conference in 1884, with two substantial publications that followed (Schulz, 1887, 1888). The research of Schulz concerned the effects of numerous toxic agents on the metabolism and growth of yeast. The findings of Schulz generated much scientific interest but also considerable contemporary scientific and medical controversy as he would claim that his findings could be used to provide the explanatory principle of the medical practice of homeopathy, which was quite prominent in Germany and other countries at that time (Calabrese, 2005, 2011). Thus, from its very beginning, the concept of biphasic dose responses became unnecessarily linked to a long standing feud between what would be come to be called "traditional" medicine and homeopathy. While Schulz never subscribed to the high dilutionist wing of the field of homeopathy, his research and concept of a biphasic dose response became very politicized and became the object of much scientific pushback in his era, with leaders in the area of pharmacology and toxicology, such as Alfred J. Clark, unfairly associating the work of Schultz with various types of medical quackery during the early decades of the 20th century. Schulz would name this phenomenon the Arndt-Schulz Law and Ferdinand Hueppe, a protege of the famous microbiologist/bacteriologist, Robert Koch, who discovered hormetic effects in bacteria, named it Hueppe's Rule (Calabrese, 2005, 2011).

Despite its controversial beginnings, the research of Schulz encouraged numerous researchers to explore the effects of low doses of chemical agents and radiation (e.g. X-rays, radionuclides) on various biological systems with particular emphasis on microbial models, including bacteria, fungi and yeast, as well with extensive studies on plants and insects (Calabrese and Baldwin, 2000a, 2000b, 2000c, 2000d, 2000e). Numerous dissertations were undertaken, especially in Germany and other European countries and subsequently in the United States. Despite this ground swell of research on biphasic dose responses it was continuously stigmatized by its initial association with homeopathy and its contentious association with the growing and powerful traditional medicine. In fact, this conflict became so historically powerful that it greatly affected the capacity of the biomedical community to explore the area of low dose induced adaptive responses throughout the 20th century, with a major transformation in such research now underway. The remainder of the 20th century witnessed the gradual progression of the scientific foundations of the concept of the biphasic dose response within the biological and medical literature. However, this area lacked organizational leadership, scientific integration and an evolutionary context which also affected its recognition and acceptance (Calabrese, 2009). However, by the 1970s the hormetic literature in the area of chemical toxicity and radiation biology became well organized and integrated with publications in leading journals and books as seen by the particularly noteworthy efforts of Anthony Stebbing and Thomas Luckey. Their leadership led to the first conference on radiation hormesis in 1985 with publication of peer reviewed papers in the journal, Health Physics in 1987, thereby providing a major stimulatory effect to the hormesis concept.

The scientific interest in hormesis dose response has expanded enormously since that first conference based upon many indices but especially seen within the contemporary scientific citations. For example, the scientific citations of hormesis or hormetic has increased from only 10 to 15 citations/year in the Web of Science database in the 1980s to nearly 20,000/year today. While the growth in the interest in the concept of hormesis and its widespread applications has displayed an impressive growth the issue of what is hormesis and how to effectively test hypotheses can still be highly problematic. The reason for this is due to the fact that hormetic dose response have highly specific quantitative features especially with respect to the amplitude of the low dose stimulation. That is, the maximum stimulation is modest, typically being only 30 to 60 % greater than the untreated control comparison group species (Calabrese and Blain, 2005, 2011). The modest increase creates experimental challenges with respect to detecting a low dose modest treatment effect from background variation. This can raise the question of whether the low dose stimulation is a real and reproducible effect or simply a manifestation of background variation. As a result of such constraints, it is necessary to place considerable focus on control group background variation with a view that lower variability will create more favorable conditions for treatment response detection. This situation also affects the number of doses used, the dose spacing and the statistical power requirement of the experiment. Further, these considerations also demand a greater requirement for study replication and the need to clarify the mechanistic foundations underlying the low dose stimulation and high dose inhibition. In practice the concept of hormesis, while well established in the early decades of the 20th century, took great advantage of the *in vitro* revolution of the 1980s in which cell culture provided greatly reduced variation, providing ease in testing far more concentrations than whole animal studies and led to marked improvements in mechanistic insights.

It is now nearly 140 years since the first presentation of a hormetic response by Schulz and much progress has been made to clarify the nature of the dose response and the low dose zone. While much of the past century of research on hormesis has been of an experimental nature there has been a parallel interest to better understand the nature of the dose response and the low dose zone with human population studies. However, the association of the hormesis concept with human subject population studies presents unique challenges. While it is well established that human cells in culture display very reproducible hormetic effects in a similar fashion as bacteria, yeast, fungi, plants, nematodes,

and animal model cells (Calabrese, 2017; Calabrese and Baldwin, 2000a, 2001; Calabrese and Blain, 2005, 2011; Iavicoli et al., 2014, 2018, 2021), the challenge has been how to study hormetic effects in human subjects that display considerable background variability and where there are numerous unknown background variables and where standard epidemiological adjustments for parameters such as for age, gender, income, race, education are very helpful but still limited and where the key features of exposure assessment can be highly problematic. The limitations of epidemiology have been long recognized in their impact on how to assess low dose effects such as hormetic effects and they have been insightfully addressed (Mundt and May, 2001). The fundamental biological concept of hormesis presents important challenges to the field of epidemiology. These challenges center around the capacity of epidemiology to reliably detect changes that are <60 % greater than the control group that reliably occur in cell culture and in highly inbred and even outbred animal models receiving standardized rearing conditions. Extensive laboratory studies have established the reproducibility (Calabrese and Blain, 2005, 2011; Calabrese et al., 2006) and mechanistic basis of the hormetic dose response (Calabrese, 2013). Yet the question may be raised as to how often highly reproducible and mechanistically based hormetic responses as shown in experimental model studies can be readily affirmed in human population studies. A fundamental practical conflict may exist between the hormetic maxima of 30 to 60 % which describes the limits of biological plasticity and the capacity of epidemiologic studies to detect such changes. The situation would be more challenging if an average increase of only half this response occurred. These effects may well be biologically reproducible in experimental systems but lost in the noise of epidemiologic studies. It is the hormetic-epidemiologic interface conundrum that leads to a higher proportion of potentially effective on drugs failing clinical trials and the failure of similar efforts to detect environmentally based adaptive responses.

What is the functional solution to this issue? The epidemiologic evaluation of low doses is a challenge whether one is trying to detect a positive or a negative response. This becomes even more challenging for humans due to heterogeneity in the population. That is, at the same dose one population subgroup may experience a benefit while another experiences an undesirable effect. Given the complexity of the low dose epidemiologic studies and the centrality of hormetic dose responses in biology and human health, it is proposed that the epidemiologic evaluation not be a "stand alone" evaluation but be integrated within a weight of evidence evaluative system in which all relevant data are included. This process has long been used in environmental risk assessment evaluations for adverse health effects. The weight of evidence model evaluation approach permits the use of the entire spectrum of relevant data and helps to recognize the significance and limitations of all experimental and population-based approaches to acquire biological understandings and optimized recommendations for human risk assessment. In this context, this conceptual article (which is the first in a series of three) addressed the challenges of defining and identifying the occurrence of hormetic responses in occupational epidemiological studies, referring specifically to radiation hormesis (RH). In addition to highlighting the main limitations and shortcomings of current epidemiological studies, this work lays the foundations for addressing in subsequent articles the methods and strategies used to evaluate RH in cells and animal models and above all the challenges of studying RH in humans, how it could possibly be done and what the expectations may be.

2. Hormesis and the risk assessment and management process in occupational medicine

Hormesis, which has rapidly become a key biological concept that, by influencing several research areas and domains (e.g., toxicology, microbiology, medicine, public health), has crucial practical consequences (Calabrese, 2018). In the field of occupational medicine (OM), the hormetic phenomenon has had and continues to have important implications that have the potential to affect most areas of the occupational safety and health (OSH) management systems, especially how it can be incorporated into prevention and protection policies and strategies to increase the protection of workers' health and safety (Calabrese, 2010). For example, with regard to chemical risk assessment the gold standard framework was set forth by the U.S. National Academy of Sciences and described in the U.S. National Research Council report "Risk Assessment in the Federal Government: Managing the Process", that point out how the risk assessment process should be based on four critical steps including hazard identification, dose-response assessment, exposure assessment and risk characterization (NRC, 1983). In this regard, the concept of hormesis has major potential significances especially for the stages one and two of the aforementioned process. In the case of hazard assessment, the hormesis concept should be used to help guide the selection of experimental model, the degree of control group disease incidence and critical study design features such as number of doses and their spacing as well as the possible inclusion of a temporal feature that may have the capacity to detect possible compensatory adaptive hormetic dose responses. In the context of the dose-response relationship since the 1930s the threshold dose-response model (Fig. 1) has been central to toxicology, pharmacology, public and occupational health regulatory agencies, influencing chemical/drug safety assessments, OSH risk assessment strategies and occupational medicine exposure standards and limits (Calabrese, 2008, 2009).

Therefore, considering the importance of this topic and the universal acceptance of this threshold model within the scientific and regulatory communities, one would expect that this dose-response model had been studied in detail, screened and validated scientifically, and that it can therefore be assumed with reasonable certainty that it is capable of providing accurate estimates of biological responses especially in the low-dose region (i.e., below toxicological thresholds). However, this is simply not true, and a growing body of scientific evidence now shows that threshold dose-response predicts responses below the estimated threshold very poorly (Calabrese and Baldwin, 2001, 2003; Calabrese et al., 2008; Calabrese, 2010). Moreover, this failure is also consistent with the publication of a large number of studies that support hormesis by showing how this dose-response model is able to make much more accurate predictions of biological responses in low-dose areas than the threshold model (Agathokleous et al., 2022). Consequently, with regard to the dose-response relationship in particular, risk modeling has basically relied on unverifiable assumptions and speculation. Indeed, the main challenge facing occupational risk assessment is the extrapolation of data from animal toxicity studies (usually from mice and/or rats to humans) that moreover use very high doses that are unrealistic compared to the low-dose exposures that workers typically experience in the workplace. Therefore, it is inevitable that this double extrapolation (from laboratory animals to humans and from high to low doses) results in significant uncertainties. Furthermore, it should also be considered that these uncertainties are also fueled by additional extrapolations, which are equally as important as those mentioned above, the extrapolation from high dose-rates (or even single-point exposures) to low and chronic dose-rates, and from cell or molecular endpoints to whole organisms (i.e., humans).

Based on the above considerations, it seems clear, and equally urgent, that there is a need to update the regulatory risk assessments on exposure and effects including in this process nonlinear dose-response models but also reconsidering potential subthreshold responses and above all abandoning the default use of linear dose-response models for all risk assessments (Agathokleous et al., 2022). This more inclusive and up-to-date approach to the latest scientific evidence is even more urgently needed when thinking about carcinogens, since for these occupational risk factors, regulatory agencies argue that the risk is directly proportional to exposure in the low-dose zone and that, therefore, there is no safe level of exposure. As we will see in the following paragraphs, this so-called linear no-threshold (LNT) dose-response model (Fig. 1) has

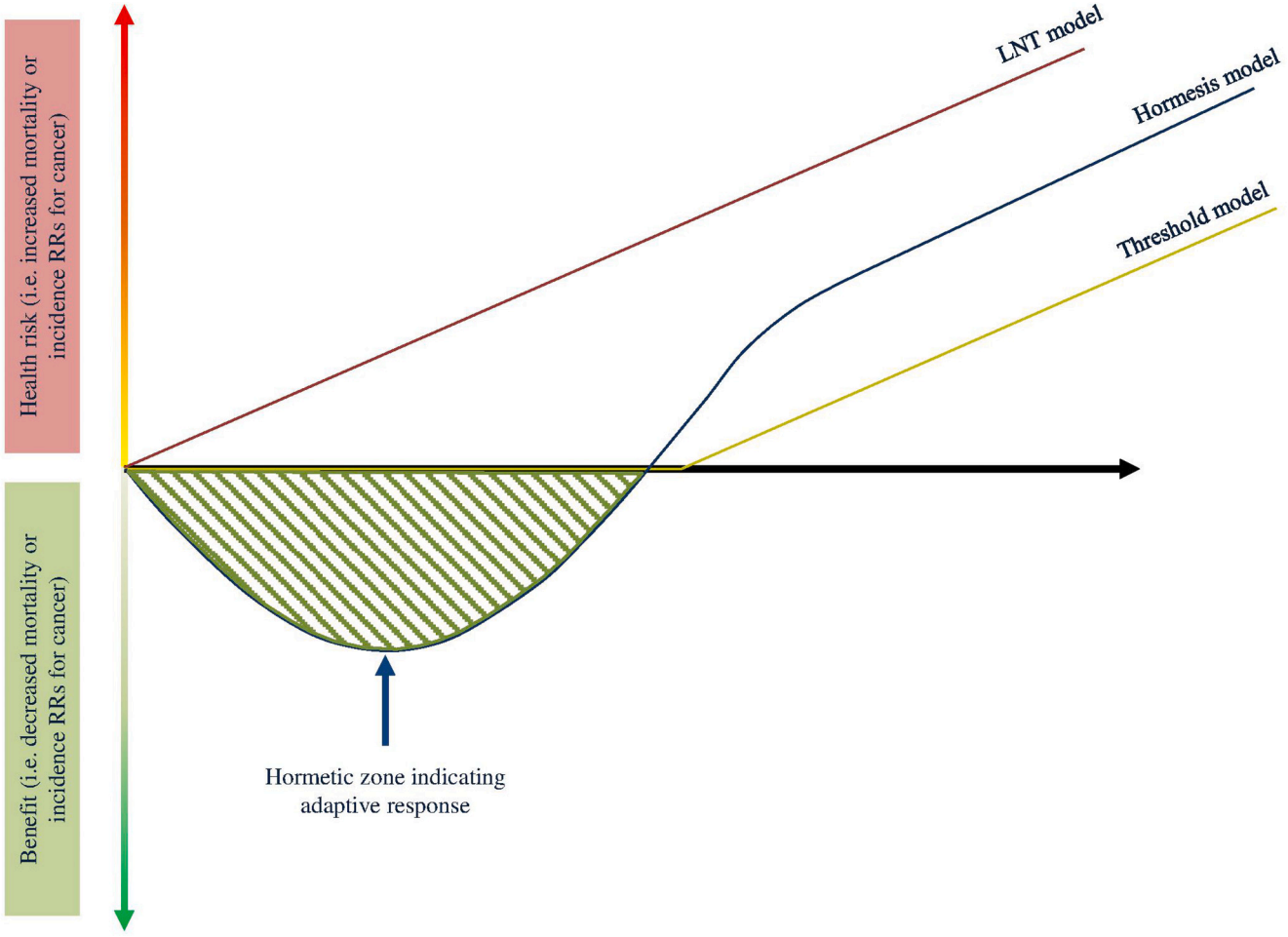


Fig. 1. Schematic illustration of different dose-response curves: linear no-threshold model, hormetic model and threshold model.

become the standard model for health risk assessment of chemical carcinogens and radiation by regulatory agencies in many countries. Therefore, in this perspective, an adequate, useful and more real-life risk assessment should be based on gathering as much useful real-world data as possible also including relevant information provided by epidemiologic studies in order to achieve the aforementioned weight of evidence model evaluation approach. In fact, occupational epidemiology by studying cohorts of workers the possible association between exposure to various occupational risk factors and the occurrence of a disease can make a substantial contribution to the risk assessment process by providing experimental studies with supplementary and complementary information especially regarding the actual exposure conditions to which workers are subjected. Unfortunately, the application of the findings of existing epidemiological studies in qualitative/quantitative risk assessment is hindered by many factors, such as lack of exact exposure information, failure to consider non-occupational exposures and other confounding factors, small sample sizes, and relatively short duration of epidemiological studies (Meijers et al., 1992; Mundt and May, 2001).

In this context, taking a cue from the risk assessment of exposure to ionizing radiation (IR) and the concept of RH, in the following section are presented the main limitations of occupational epidemiological studies (which prevent their full and conscious use both in the identification/definition of the hormetic response and consequently in the complex process of risk assessment at low exposure doses) and at the same time are provided possible approaches to improve their impact in the risk assessment process.

3. The linear no-threshold dose-response model and the radiation hormesis concept

IR is an extremely important occupational risk factor to which about 30 million workers, employed in various work environments such as healthcare sector, research laboratories, nuclear power plants, nuclear weapons production facilities, air and space transportation, are professionally exposed (Cioffi et al., 2020; Wakeford, 2009). The International Commission on Radiological Protection (ICRP) recommends limits on occupational radiation exposure of 20 mSv per year effective dose averaged over defined 5-year periods and not exceeding 50 mSv in a single year (ICRP, 2007). Usually, in workplaces where IR are used, this type of exposure is characterized as being chronic and to low-doses, where low-dose radiation exposure is defined as $\leq$100 mSv and the low-dose radiation rate is $\leq$6 mSv per hour (ICRP, 2007). In this regard, most epidemiological data available today and published in the scientific literature substantially support the hypothesis that no detrimental effects should be observed at these exposure doses (Vaiserman, 2010; Vaiserman et al., 2018) and in any case, even to be more cautious, it can be safely stated that the evidence on the potential adverse health effects of IR low-doses is at least controversial and conflicting.

Nonetheless, throughout the world, the framework for adopting prevention and protection policies against IR, in order to safeguard the health of both occupationally exposed subjects and the general population, is entirely built on the LNT model (Averbeck et al., 2018; Seong et al., 2016) whose fundamental assumption is that IR (no matter how low the exposure dose is) would be able to induce dose-proportionally damaging effects, especially cancers (Fig. 1). Indeed, the LNT assumes

that the risk of cancer is directly proportional to the number of cells damaged by IR, that is, in other words, that more IR passes through the nuclei of cells, the more DNA damage will occur and thus the greater the biological response will be (Jargin, 2020). Then, according to this hypothesis, based on the dual dogmas of DNA and target theory (that is the concept that the biological effects of IR are the result of ionization induced at sensitive targets in a cell such as DNA), and considering the independent and random action of IR, any dose no matter how small could cause a DNA strand break inducing a mutation and thus ultimately triggering the process of carcinogenesis (Mothersill and Seymour, 2022). This model was recommended in 1956 (NAS/NRC, 1956, 1960) and it is essentially based on and derived from the epidemiological data related to atomic bomb survivors that are deemed the "gold standard" for estimating the cancer risk correlated to IR exposure (Doss, 2013; Hall and Brenner, 2008). In addition, other epidemiological data such as those from survivors of the Chornobyl disaster and other populations with documented high exposures to IR are generally used to affirm the absence of a threshold dose and then support the LNT model as done for example by 2006 National Academy of Sciences Biologic Effects of Ionizing Radiation (BEIR) VII report (NRC, 2006; Parsons, 1990).

Therefore, the main issue with the use of LNT extrapolation model lies in the fact that it assumes, even at low or very low-doses of exposure the occurrence of adverse biological effects, that are thus linearly related to dose (Calabrese and Baldwin, 2000b, 2000c; ICRP, 2007; Seong et al., 2016). However, it is well recognized that the use of the LNT extrapolation model to predict detrimental effects in the low-dose region basing on those observed at higher exposure doses is characterized by significant uncertainties (Cardarelli 2nd and Ulsh, 2018; Jeong et al., 2010; Scott, 2018) and there continues to be a substantial disagreement in the scientific community as to whether this model should be used since several studies have challenged, if not outright refuted, its validity (Pennington and Siegel, 2019). Indeed, accumulating evidence demonstrated that the biological effects induced by low-dose IR are significantly different from those observed at high exposure doses (Ji et al., 2019) and over the past few years several hundred studies (*in vitro*, *in vivo* and *ex vivo*), investigating the possible biological effects related to such exposures, suggested beneficial or no effects (Calabrese and O'Connor, 2014; Devic et al., 2020; Luckey and Lawrence, 2006; Pollycove and Feinendegen, 2001; Shibamoto and Nakamura, 2018). In contrast to the LNT paradigm, these findings support the concept of RH, which has long been known, having been introduced in the early 1980s by Luckey (1980, 1991) to describe the bio-positive effects (in terms of animal growth, development, health and longevity) linked to low-dose IR exposures (Baldwin and Grantham, 2015). In this regard, convincing evidence of the occurrence of these inducible adaptive responses in several organisms, following exposure to IR, has been extensively reviewed by Calabrese and Baldwin (2000b). According to the hormesis phenomenon "any physiological effect that occurs at low-doses cannot be anticipated by extrapolating from toxic effects noted at high doses" (Sagan, 1987) which is precisely what happens in IR risk assessment using the LNT extrapolation model. Thus, RH is characterized by low-dose IR stimulation and high-dose IR inhibition of living systems (Fig. 1) and essentially theorizes their ability to express/upregulate adaptive mechanisms to cope with low-doses of IR thus generating a biological stress-response strategy that, by improving functionality and/or tolerance to more serious challenges, increases their resilience.

Interestingly, several epidemiological studies, analysing mortality and morbidity rates (especially for cancer diseases) of different working populations exposed to IR (e.g., healthcare, nuclear and industrial irradiation and aircrew workers) have observed lower disease incidence than the general population (Seong et al., 2016; Shibamoto and Nakamura, 2018; Vaiserman, 2010; Vaiserman et al., 2018) and frequently the analysis of the dose-response curve would suggest the presence of a possible hormetic phenomenon thus supporting the hypothesis that, at low-doses, IR may induce an adaptive response rather than causing adverse health effects in workers. As stated previously, from an epidemiological point of view, the research, definition, and detection of hormetic responses in occupational studies would be particularly important for the risk assessment and management process but unfortunately there are several key limitations that often prevent the possibility of validly detecting and interpreting hormesis (Mundt and May, 2001). Of these, the main issues relate to study sample size and statistical power, time dependency, assessment of exposure, definition of accurate exposure doses and appropriate evaluation of confounding risk factors (Mundt and May, 2001).

4. The issue of exposure dose definition and the use of multiple exposure doses

It is important to highlight the fact that hormesis phenomenon has been increasingly observed, thoroughly investigated and broadly accepted over the past two decades (Agathokleous and Calabrese, 2019, 2020; Calabrese, 2015) thanks mainly to the extraordinary improvements in chemical analysis and the adoption of large-scale testing which have increasingly allowed low doses/concentrations and many doses/concentrations to be tested in cell culture and experimental studies, whereas previously dose-response assessment in toxicology relied primarily on the use of a few extremely high doses and the subsequent application of bio-statistical models to estimate responses to low doses/dose rates, often many orders of magnitude lower than those tested (Calabrese et al., 2023a, 2023b). In fact, the peculiar quantitative features of the hormesis response usually require that the investigated agent be tested over a broad range of doses and, at the same time, also considering that hormetic dose-response is a plausible overcompensation to an initial disruption of homeostasis, it should be necessary include also repeated measures or time component (i.e. different lengths of exposure and several time points for assessment of the data) (Iavicoli et al., 2014, 2018, 2021). Unfortunately, in the context of epidemiological studies it is not possible to establish these rigorous methodological criteria a priori, and very often it is not only difficult to identify appropriate ranges of exposure doses but also the very accurate definition of exposure to the agent being evaluated is a rather challenging task. Indeed, few epidemiological studies are based on direct measurements of exposure and consequently surrogate measures are often used (Mundt and May, 2001).

In this regard, studies that have investigated the possible adverse effects of exposure to IR low doses are also no exception since different proxies of IR exposure were used. For example, in several studies performed on the healthcare workers the "year first worked" is often employed as a surrogate of cumulative radiation exposure on the assumption that in past years (several decades ago) exposure doses were likely to be much higher than in more recent years since, in the meantime, much more stringent radiation protection regulations, that have significantly reduced exposure levels, have been enacted and enforced (Fig. 2). In addition, other indirect measures of exposure related to work history such as "graduation year", "total years worked", "number of years worked before a specific year", "number of flights" are often called into question in studies of both healthcare, nuclear and flight crew workers (Berrington et al., 2001; Berrington de González et al., 2016; Cha et al., 2020; Hauptmann et al., 2003; Linet et al., 2017; Liu et al., 2014; Matanoski et al., 1987; Mohan et al., 2003). Although these surrogate indicators might provide a rough estimate of the exposure dose which can be somewhat useful for assessing mortality and/or incidence relative risks (RRs) of a given pathology, it is quite obvious that they are unfortunately of limited and unreliable use when the aim is to evaluate the dose-response curve and above all try to identify, within this, the possible presence of a hormetic response. Indeed, as pointed out by Mundt and May (2001), the dose groupings based on the aforementioned surrogate measures might more accurately reflect groups of workers among which there is an increasing probability of exposure but nevertheless it often remains unknown whether or to what extent

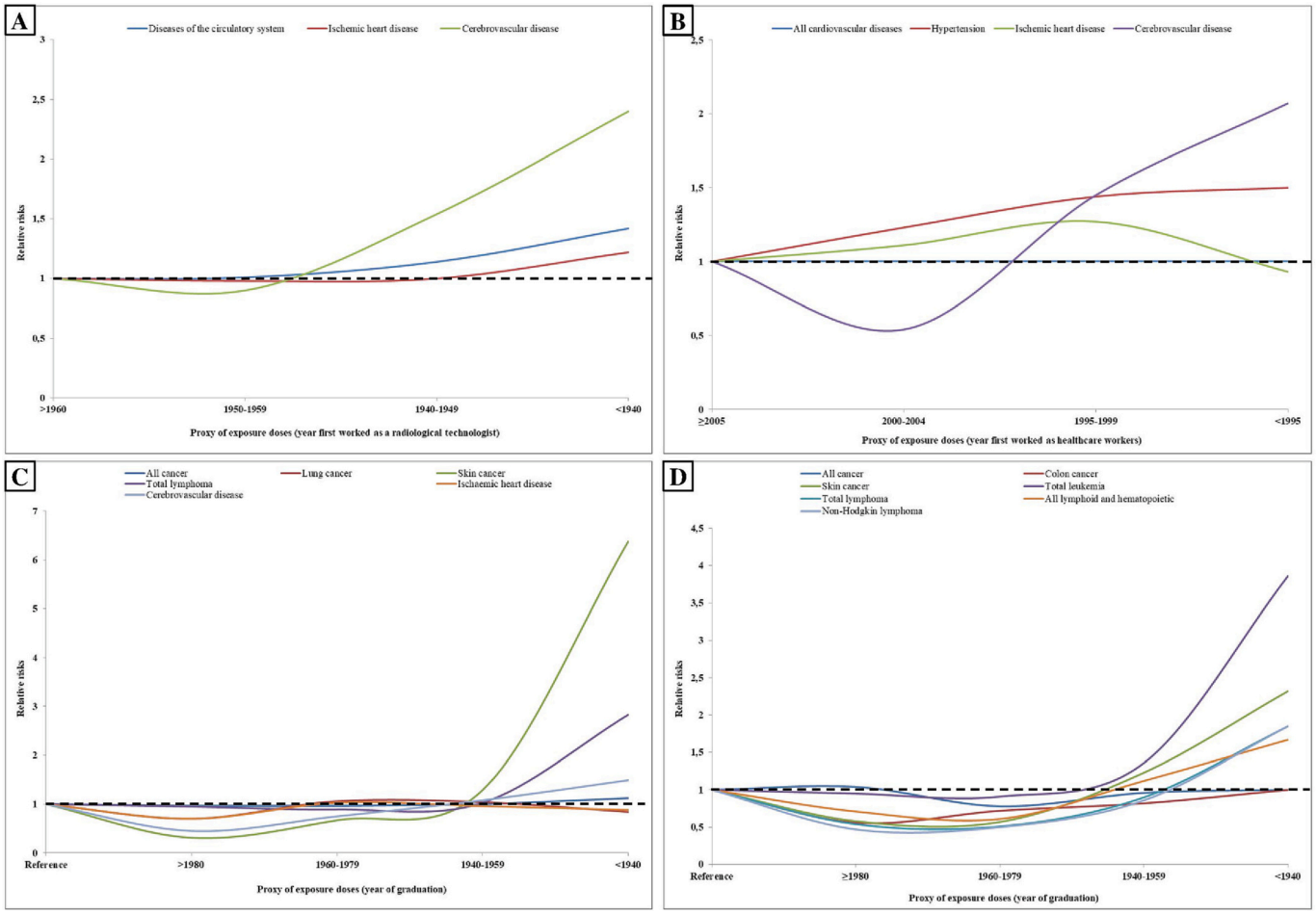


Fig. 2. Examples of supposed hormetic responses observed in healthcare workers using surrogates of exposure doses.
(A) Mortality relative risks for circulatory system disease, ischemic heart disease and cerebrovascular disease (Hauptmann et al., 2003); (B) Incidence relative risks for all cardiovascular disease, hypertension, ischemic heart disease and cerebrovascular disease (Cha et al., 2020); (C) Mortality relative risks for all cancer, total lymphoma, cerebrovascular disease, lung cancer, skin cancer, ischaemic heart disease (Berrington de González et al., 2016); (D) Mortality relative risks for all cancers, skin cancer, total lymphoma, non-Hodgkin lymphoma, colon cancer, total leukemia, all lymphoid and hematopoietic cancers (Linet et al., 2017).

exposure occurred. However, the possible presence of a hormetic dose-response curve can also be traced in some epidemiological studies (conducted mainly on nuclear workers) in which instead the definition of the exposure dose, and the subdivision of the cohorts in different categories with increasing exposure, was accurately performed on the basis of dosimetry data provided by personal dosimeters worn by workers (Azizova et al., 2010; Boice Jr et al., 2011; Gillies et al., 2017; Grellier et al., 2017; Gyuleva et al., 2015; Iwasaki et al., 2003; Jeong et al., 2010; Metz-Flamant et al., 2012; Sponsler and Cameron, 2005; Tokarskaya et al., 1997; Zablotska et al., 2004). Moreover, in addition to the IR external dose, individual doses for different organs from various internally incorporated radionuclides have also been reconstructed using bioassay data, such as urine and faecal analysis (Grellier et al., 2017), or whole body counters data (Jeong et al., 2010). Nevertheless, even regardless of having reliable exposure data available, the probability of effectively identifying a hormetic effect is still inhibited by the fact that in most cases the dose range evaluated is rather narrow (mainly restricted to two or three doses). Consequently, the assimilation of a low or high exposure condition with a single exposure dose might prevent the possibility to observe the hormetic area or the inhibitory part of the classic hormetic dose-response model, respectively (Fig. 3).

Another rather important exposure-related issue that is common to these epidemiological studies is the lack of a proper control group. For instance, in most of the studies that have analysed the mortality and/or incidence rates for cancer or other non-malignant diseases, the excess risk in workers is estimated by referring to the relative mortality and/or incidence rates of the general population. Exceptions to this generalized approach and worthy of mention are the studies carried out by Berrington de González et al. (2016) and Sponsler and Cameron (2005). Indeed, in order to minimize potential selection biases, in the first study, which investigated mortality rates in radiologists, psychiatrists were recruited as control group not exposed to IR, whereas in the second the standardized mortality ratios of shipyard workers exposed and not exposed to IR were compared. Furthermore, it should be noted that usually in the evaluation of the adverse health effects as a function of increasing doses and in the assessment of the dose-response shape the referent group is often identified in the lowest-dose exposure group of workers (Azizova et al., 2010; Grellier et al., 2017; Tokarskaya et al., 1997; Zablotska et al., 2004). However, from a hormetic point of view, this approach is questionable since it eliminates from the dose range exposure the lowest dose which is precisely the one at which, according to the hormesis model, one expects to observe the appearance of the adaptive response. Therefore, on the whole, if we consider together this last problem and the previous one related to the narrowness of the dose range in the low-dose exposure region it is plausible to speculate that these methodological limitations may actually hinder the detection of hormesis by "hiding" or "camouflaging" possible hormetic areas. In any case, an appropriate choice of the reference or control group seems to be a critical element in order to be able to adequately detect, especially at low or very low doses of exposure, a trend in the dose-response curve of

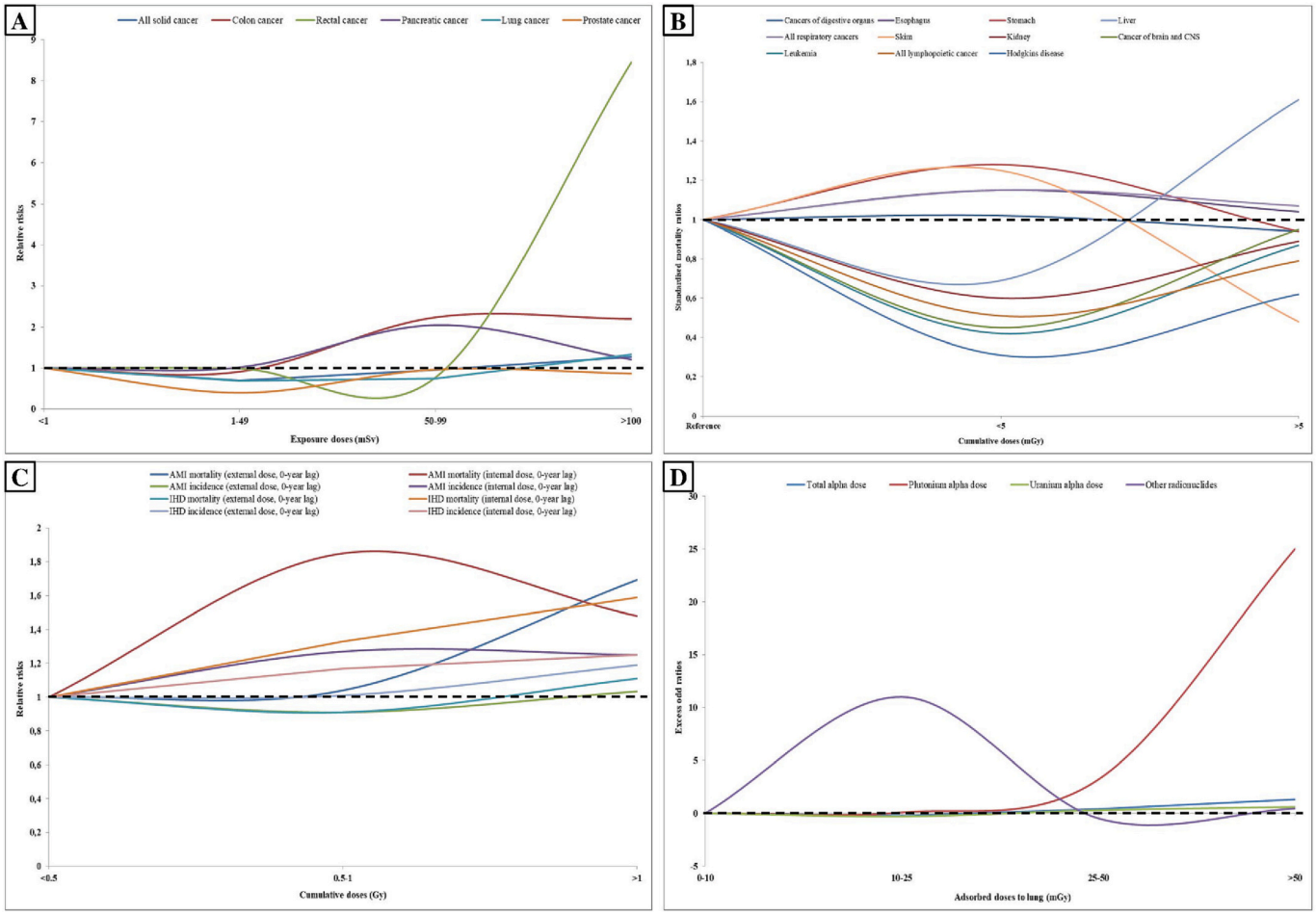


Fig. 3. Examples of supposed hormetic responses observed in nuclear workers using narrow dose ranges.
(A) Mortality excess relative risks for all solid cancer, colon, rectal, pancreatic, lung and prostate cancers (Zablotska et al., 2004); (B) Standardized mortality ratios for digestive organs cancers, all respiratory cancers, leukemia, Hodgkin disease, oesophagus, skin, all lymphopoietic, stomach, kidney, liver, brain and CNS cancer (Sponsler and Cameron, 2005); (C) Mortality and incidence relative risks for ischemic heart disease (IHD) and acute myocardial infarction (AMI) both for external and internal dose (Azizova et al., 2010); (D) Mortality excess odds ratios lung cancer for total, plutonium, uranium and other radionuclides alpha dose (Grellier et al., 2017).

a hormetic, linear, or any other nature such as that suggested for example at the cellular level by Burlakova (2000) in some experimental studies (Sacks et al., 2016).

5. Radiation hormesis and the concept of dose

Hormetic dose response relationships are reported at the cell, organ and individual level. Even at the cell level in *in vitro* studies, the response is not that representing a single cell, but a cell that has been cultured with many thousands of similar cells, that interact, display cell to cell communication, perform bystander communications and other interactive activities. These cells often of the same cell type although this is not always the case. At the level of organ, there is more complexity due to the presence of a wide range of cell types and their unique spatial orientation and integration and their relationship to the circulatory system with the flow of oxygen/removal of carbon dioxide which also affects nutrient, hormonal and other agent pharmacokinetics. The situation is even more complex with an entire individual. However, hormetic mechanisms at the cell level have been well characterized at the level of receptor and cell signaling pathway and confirmed by the use of receptor and pathway inhibitors (Calabrese, 2013). These agents act in cell culture on a population of cells. There is some degree of variability across cells to the agent concentration at the intracellular sites of action. What is observed in experimental evaluation is the net integration of the cell population responses. In more complex organs and whole organism situations is that the hormetic effect occurs in a similar general fashion. For example, neurotrophic hormetic agents that pass the blood brain barrier get distributed to target sites such as the hippocampus. After reaching the hippocampus, cell populations within that target concentrations may become hormetically stimulated to enhance neural stem cell proliferation. This process occurs in a single individual and would be repeated in others comprising an epidemiologic sample.

In the case of IR it can affect anti-inflammatory responses via a range of endpoints within a relatively narrow dose range. This may include the activation of NrF2 which would then lead to the activation of a plethora of antioxidant enzymes (Calabrese and Kozumbo, 2021). In addition, IR within a similar dose range often affects the reprogramming of macrophages from a M1 to M2 state. These cells then migrate to zones of inflammation to reduce the inflammatory process. Thus, the concept of cell, organ, and individual hormetic responses are integrated with the key action occurring at the cellular level in each case. However, the hormetic optimal dose that occurs at the cell level will be necessarily integrated into the overall organ and individual dose framework. When dosing is reported in cellular, organ or individuals/epidemiologic studies this arrangement will lead to the occurrence of biphasic dose responses at each of the three levels of biological organization. However, the specific optimal hormetic dose range at these different levels of biological organization would need to reflect the system within which

the study is conducted. Yet, the dosing at the cell level in each of the three cases would be expected to the similar. In this context, with regard to RH and its relationship to the concept of dose, a further major challenge that needs to be resolved in order to have adequate tools available to identify and define the occurrence of this phenomenon in epidemiological studies, is that of correctly framing, distinguishing and assessing the interactive effects resulting from internal and/or external exposures. Indeed, the concept of dose in epidemiological studies does not distinguish between internal ionization processes in cells from either chemically bound radionuclides or from hot particles (Busby, 2021). For example, Strontium-90 dose is calculated in the same way as an X-ray dose and the quantity dose is assumed to have the same effect on cancer risk whatever the source of exposure (Busby, 2022). However, this approach is considered inappropriate by some authors according to whom the proper quantity dose for radiation risk studies is the average ionization at or near the nuclear DNA at the time of replication of the cell (Busby, 2021, 2022). These and other aspects (such as for example the International Commission on Radiological Units and measurements choice of the quality factor "Q" multiplier of 20 for radioactive alpha particles to change the physical dose in Grays into an "Effective Dose" in Sieverts) of the dose concept should be given careful consideration in the analysis of epidemiological studies involving nuclear workers (where internal exposure takes on a particularly prominent role) as they can greatly influence the construction of the dose axis in the dose-response relationship (Busby, 2022).

6. The assessment of confounding factors

In the context of occupational exposure to IR, most epidemiological studies aim at assessing, mainly by estimating disease incidence or mortality RRs, potential adverse health effects related to IR exposure. Since IR high doses is a recognized risk factor for cancer, it is not surprising that particular attention has been dedicated to the analysis of cancer pathologies. However, in this regard, cancer is a multifactorial disease to whose etiopathogenesis contribute numerous risk factors of different types (e.g. unmodifiable intrinsic risk factors, modifiable exogenous/external risk factors such as viruses and occupational exposures, lifestyle factors such as smoking, hormone therapy, nutrient intake, physical activity and endogenous risk factors such as immune system, metabolism, and hormone levels) (Wu et al., 2018). Therefore, since each of the above parameters may to some extent play a role in the occurrence of a specific cancer, it is clear that when attempting to accurately and reliably establish the strength of an association between a specific risk factor (in our case, IR) and the disease of interest, it is necessary to perform the appropriate statistical analyses by also taking into consideration these potentially significant confounding factors.

Indeed, confounding factor issues are important in any epidemiological study, but they are even more relevant in low-dose radiation studies since even a modest degree of confounding might significantly skew study findings thus yielding misleading interpretations (NRC, 2012). In practice, the failure to take into account at least the most important and well-known (for their association with cancer such as smoking) confounding factors may result in the calculation of incorrect risk estimates that may therefore exaggerate or nullify the true degree of association (Hajian, 2012; NRC, 2012). Typically, this problem does not arise when investigating the health effects possibly associated with IR exposure to high doses, since in these cases the influence, even of particularly relevant confounders, is expected to be considerably weaker than that of the radiation itself. In contrast, in the assessment of adverse outcomes related to low-dose IR, the effect of confounding factors on the interpretation of results could be as great or greater than the size of the radiation effect (which, in fact, if it exists, may be expected to be small) (NRC, 2012). Thus, without adequate information on these confounding factors, which should be used in statistical analyses to make adjustments in risk estimation, there is an important source of uncertainty that can hamper the interpretation of effects in low-dose studies (Mundt and May, 2001). Unfortunately, in many studies this information on the numerous confounding factors is missing. For example, due to the above considerations, it is evident that the lack of data on the individuals' smoking history (e.g., number of cigarettes smoked per day, age of smoking initiation, years of smoking) compels careful consideration of the results relating to cancers with a strong association with smoking (i.e., lung cancer) (Berrington de González et al., 2016; Pinkerton et al., 2012; Preston et al., 2016; Matanoski et al., 1987; Zablotska et al., 2004; Zeeb et al., 2002). In this regard, the lung cancer RR correlated to cigarette smoking normally exceeds 10 and, on the other hand, the RR associated with exposure to IR high doses rarely exceeds 2 (Pierce et al., 2005).

7. Biological and molecular mechanisms of action underlying radiation hormesis

In the preceding paragraphs, we discussed how various limitations (e.g., lack of exposure doses, limited availability of dose ranges, selection biases and confounding factors) found in epidemiological studies have an important influence on the accuracy of risk estimations. Therefore, considering these uncertainties and the relative difficulties in detecting with confidence, within the framework of epidemiological studies, the presence of the hormetic phenomenon in the presence of low-doses of IR exposure, the need to define the possible molecular mechanisms of action underlying the expression of this adaptive response becomes even more relevant. Indeed, gaining knowledge of the biological mechanisms linking an exposure to a specific response is a necessary component of the evidentiary process in establishing a direct causal relationships (NRC, 2012) and in this regard experimental studies (i.e. *in vitro* and *in vivo* studies) are crucial. However, epidemiological studies can also play an appreciable role in this area of research and, not surprisingly, in recent years there has been a growing interest in this field, owing in part to the decisive boost given to molecular epidemiology by omics techniques (Smith et al., 2011). Therefore, from this perspective, in order to improve our current knowledge of the mechanisms underpinning RH, it would be desirable to better integrate the results provided by experimental and epidemiological studies with a view to applying the multidisciplinary approach of the molecular epidemiology and system biology. Indeed, in this context, the use of different omics techniques in epidemiological studies has the potential to advance the ability of molecular epidemiology to more broadly explore exposure-response relationships and, at the same time, gain insight into the fundamental underlying molecular mechanisms of action of hormetic responses (Smith et al., 2011). On the other hand, the need to arrive at an integrated research model that takes into account and makes coordinated use of both the results of basic science studies in radiation biology and epidemiologic studies on adverse health effects induced by IR low doses has also been recognized by the US National Council on Radiation Protection and Measurements (NCRP) which in its commentary No. 24 (NCRP, 2015) suggested several proposals and strategies to fill the gaps towards this integrated approach such as for example to make a more extensive use of informative biomarkers of exposure and early cellular effects and bioindicators of adverse health outcomes. Furthermore, on the same perspective and even more recently, NCRP prepared an additional document, the commentary No.186 in which available or potential approaches for combining data from epidemiology and radiation biology studies into models for predicting low dose/low dose rate IR risks are described (NCRP, 2020). The integration of epidemiology and informative radiation biology data in biologically-based dose-response models is deemed to be a viable approach but currently, in this regard, there are several research needs such as that of identifying adverse outcome pathways and key events for radiation-induced cancers and/or non-cancer diseases related to specific exposure scenarios, especially at low doses and low dose rates.

A practical example of this relates to the ability of IR to cause oxidative stress and the role that reactive oxygen species (ROS)

detoxification systems might play in inducing the hormetic response (Fig. 4). In fact, one of the first-line defensive mechanisms to counteract the damaging effects of IR is the activation of systems to facilitate scavenging ROS (Kabilan et al., 2020). In this regard, several experimental studies showed that an enhanced antioxidant activity, sustained by an overexpression of manganese superoxide dismutase, catalase and glutathione peroxidase and by a simultaneous suppression of superoxide anion generation, has been associated with the beneficial effects of IR low-doses (Bravard et al., 1999; Chen et al., 2015; de Toledo et al., 2006; Paraswani et al., 2018; Yamaoka, 2006). However, the results of epidemiological studies are much more nuanced and often conflicting, and above all (due to the lack of several exposure doses) rarely allow the trend of the dose-response curve to be assessed, especially at low doses (Ahmad et al., 2016; Durović et al., 2008; Russo et al., 2012). In this regard, a recent study, investigating the glutathione levels in healthcare workers exposed to different IR doses, observed a likely hormetic response with an increase of GSH content with annual effective dose from 0 mSv to 0.5 mSv and a decrease after 0.5 mSv (Fig. 4) (Tian et al., 2022).

Thus, considering that replication of findings across studies is one of the most powerful criteria for establishing a direct relationship, the carrying out of appropriate epidemiological studies (taking due account of the critical issues previously mentioned) that are not only dedicated to estimating RRs but also exploit the potential of molecular epidemiology would be extremely useful in confirming the hypotheses driven by experimental studies regarding RH. Conversely, by exploiting the advantages of omics techniques to obtain a high-throughput quali-quantitative characterization of several pools of biological molecules such as genes, proteins and metabolites, epidemiological studies, directly evaluating in workers exposed to IR how the levels of these molecules change according to different exposure doses, could provide valuable insights on the potential mechanisms of action involved in RH which should then be thoroughly studied and confirmed by experimental studies (Chu et al., 2019; López de Maturana et al., 2016; Subedi et al., 2022). Since it is now widely accepted by most low-dose radiobiologists that in a traditional IR dose-response curve, there is a breakpoint where the ratio of the dominant mechanisms shifts away from those primarily based on signaling (low-dose-region) to DNA-target ones (high-dose area) (Belli and Tabocchini, 2020; Shuryak et al., 2021), it follows that in this research area, especially with regard to transcriptomics, there is enormous potential for improvement in understanding, identifying and defining the RH mechanisms.

8. Conclusions

Current preventive measures and occupational exposure limits for controlling exposures to IR are established on the basis of the LNT extrapolation model, according to which, by observing the association between adverse effects and IR high doses, it is inferred that any dose, no matter how small, should be harmful to humans and then cause cancer, for example. However, it should be noted that, as stated by the NCRP, "…essentially no human data can be said to prove or even to provide direct support for the LNT concept with its implicit uncertainties of non-threshold, linearity and dose rate independence with respect to risk." (*NCRP*, 1995). More recently, several documents issued by different international authorities involved in radiation safety protection declared that biological responses exerted by low-dose radiation are substantially different from those correlated to high-dose exposure, having then different dose-response relationships (NRC, 2006; Tubiana, 2005; UNSCEAR, 2012; Valentin, 2005). On the other hand, this diversity is also supported by the results of studies that have investigated the molecular mechanisms underlying the effects induced by IR, since at high doses they would be supported by the DNA target theory, whereas at low doses signaling and regulation of mRNA translation control mechanisms would be far more relevant (Kabilan et al., 2020; Mothersill and Seymour, 2022; Sutou, 2022). Therefore, on the basis of these data the above mentioned documents claimed that IR low-dose effects cannot be concluded to be harmful to human health (NRC, 2006; UNSCEAR, 2012).

Similarly, the findings of occupational studies, suggest that statistically significant adverse health effects induced by occupational exposure to IR low-doses have not frequently been observed (Devic et al., 2020; Seong et al., 2016; Vaiserman, 2010; Vaiserman et al., 2018). On the contrary, a common trend is observed in these studies, showing in most of the occupational populations examined a lower mortality (or even incidence) from all causes (and especially from cancer) than in the general population or (more rarely) in workers not exposed to IR. In some studies these findings have been explained by calling into question the presumed "healthy worker effect" but no quantitative estimation of this effect has been performed and, as argued by some authors, in many cases this effect is not plausible (Fornalski and Dobrzynski, 2009; Vaiserman et al., 2018). Then, considering that the statistically significant reduction in expected deaths has been demonstrated also in nested-case-control studies (where the study design is aimed at eliminating the healthy worker effect) (NIOSH, 2001), the occurrence of hormesis should be considered as a concrete and real possibility in explaining and interpreting these results.

In this perspective, also taking into consideration the important social, ethical and economic implications deriving from the adoption of current regulatory policies based on an uncritical acceptance of the LNT model, it is necessary and urgent to include in the radiological protection decision-making process the concept of RH. For example, in this regard it was recently argued by Scott (2021) that poorly-designed epidemiologic studies, as with some that evaluated cancer risks associated to IR low-dose exposure, using various misinforming procedures, can fuel a radiation phobia leading to the adoption of decidedly harmful measures such as in the cases of the Fukushima and Chornobyl nuclear accidents. Nowadays, the increasingly emerging scientific evidence questioning the validity of the LNT model by highlighting the presence of an adaptive response of the human being when exposed to IR low-doses supports an unavoidable and timely reconsideration of the current regulation of IR. However, in this context it is crucial to that the concept of RH be properly considered in the context of both occupational medicine and public health. This would be accomplished by conducting further studies which, avoiding the main methodological limitations (e.g., assessment and definition of accurate exposure doses, inclusion of adequate control groups, appropriate evaluation of confounding risk factors) highlighted in this conceptual paper, can further assess epidemiologically-based RH hypotheses. In this regard, future studies should provide an integrated and multidisciplinary study design which, by exploiting the potential of omics techniques and systems biology approaches, is expected to significantly improve our understanding of causative molecular mechanisms of action underlying the RH phenomenon.

Funding

EJC acknowledges long-time support from the US Air Force (AFOSR FA9550-19-1-0413) and ExxonMobil Foundation (S18200000000256). The U.S. Government is authorized to reproduce and distribute for governmental purposes notwithstanding any copyright notation thereon.

CRediT authorship contribution statement

Ivo Iavicoli: Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Luca Fontana:** Investigation, Visualization, Writing – original draft, Writing – review & editing. **Carolina Santocono:** Investigation, Visualization, Writing – original draft, Writing – review & editing. **Davide Guarino:** Investigation, Visualization, Writing – review & editing. **Martina Laudiero:** Investigation, Writing – review & editing. **Edward J. Calabrese:** Conceptualization,

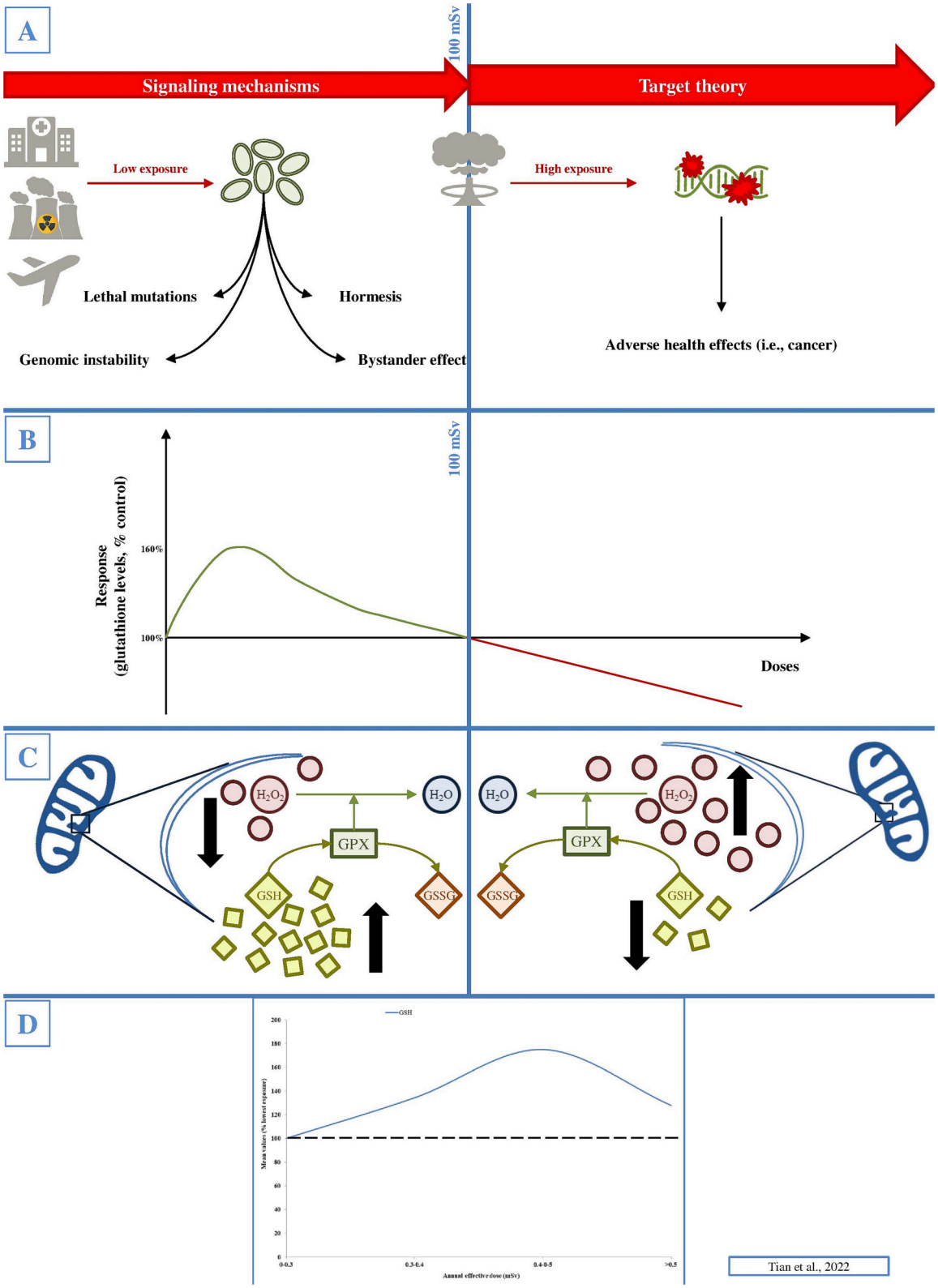


Fig. 4. Schematic representation of the possible role of ROS scavenging systems (with particular reference to the glutathione peroxidase system) in the induction of the hormetic response.

A) It is recognized and accepted that in a traditional dose-response relationship for exposure to IR, there is a breaking point where the balance of the dominant molecular mechanisms of action shifts from those primarily based on signaling to those centered on DNA damage; B) Graphical representation of a hypothetical hormetic dose-response curve relating to glutathione (GSH) levels; C) At low doses of exposure to IR, a possible molecular mechanism of action, underlying the adaptive response of cells, would be an increased availability of GSH, which in turn would facilitate the scavenging of ROS by allowing faster and more efficient metabolization by glutathione peroxidase (GPX) of hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen. As the exposure dose increases, however, the adverse effects override the adaptive mechanisms, leading to reduced GSH levels and increased oxidative stress; D) Mean values (% of lowest exposure group) of GSH observed in healthcare workers exposed to different annual effective doses (mSv) of IR (Tian et al., 2022).

Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

Agathokleous, E., Calabrese, E.J., 2019. Hormesis: the dose response for the 21st century: the future has arrived. Toxicology 425, 152249.

Agathokleous, E., Calabrese, E.J., 2020. A global environmental health perspective and optimisation of stress. Sci. Total Environ. 704, 135263.

Agathokleous, E., Barceló, D., Aschner, M., Azevedo, R.A., Bhattacharya, P., Costantini, D., Cutler, G.C., De Marco, A., Docea, A.O., Dórea, J.G., Duke, S.O., Efferth, T., Fatta-Kassinos, D., Fotopoulos, V., Ginebreda, A., Guedes, R.N.C., Hayes, A.W., Iavicoli, I., Kalantzi, O.I., Koike, T., Kouretas, D., Kumar, M., Manautou, J.E., Moore, M.N., Paoletti, E., Peñuelas, J., Picó, Y., Reiter, R.J., Rezaee, R., Rinklebe, J., Rocha-Santos, T., Sicard, P., Sonne, C., Teaf, C., Tsatsakis, A., Vardavas, A.I., Wang, W., Zeng, E.Y., Calabrese, E.J., 2022. Rethinking subthreshold effects in regulatory chemical risk assessments. Environ. Sci. Technol. 56, 11095–11099.

Ahmad, I.M., Temme, J.B., Abdalla, M.Y., Zimmerman, M.C., 2016. Redox status in workers occupationally exposed to long-term low levels of ionizing radiation: a pilot study. Redox Rep. 21, 139–145.

Averbeck, D., Salomaa, S., Bouffler, S., Ottolenghi, A., Smyth, V., Sabatier, L., 2018. Progress in low dose health risk research: novel effects and new concepts in low dose radiobiology. Mutat. Res. Rev. Mutat. Res. 776, 46–69.

Azizova, T.V., Muirhead, C.R., Druzhinina, M.B., Grigoryeva, E.S., Vlasenko, E.V., Sumina, M.V., O'Hagan, J.A., Zhang, W., Haylock, R.G., Hunter, N., 2010. Cardiovascular diseases in the cohort of workers first employed at Mayak PA in 1948-1958. Radiat. Res. 174, 155–168.

Baldwin, J., Grantham, V., 2015. Radiation Hormesis: historical and current perspectives. J. Nucl. Med. Technol. 43, 242–246.

Belli, M., Tabocchini, M.A., 2020. Ionizing radiation-induced epigenetic modifications and their relevance to radiation protection. Int. J. Mol. Sci. 21, 5993.

Berrington de González, A., Ntowe, E., Kitahara, C.M., Gilbert, E., Miller, D.L., Kleinerman, R.A., Linet, M.S., 2016. Long-term mortality in 43 763 U.S. radiologists compared with 64 990 U.S. Psychiatrists. Radiology 281, 847–857.

Berrington, A., Darby, S.C., Weiss, H.A., Doll, R., 2001. 100 years of observation on British radiologists: mortality from cancer and other causes 1897-1997. Br. J. Radiol. 74, 507–519.

Boice Jr., J.D., Cohen, S.S., Mumma, M.T., Ellis, E.D., Eckerman, K.F., Leggett, R.W., Boecker, B.B., Brill, A.B., Henderson, B.E., 2011. Updated mortality analysis of radiation workers at Rocketdyne (atomics international), 1948-2008. Radiat. Res. 176, 244–258.

Bravard, A., Luccioni, C., Moustacchi, E., Rigaud, O., 1999. Contribution of antioxidant enzymes to the adaptive response to ionizing radiation of human lymphoblasts. Int. J. Radiat. Biol. 75, 639–645.

Burlakova, E.B., 2000. Low Doses of Radiation, Are they Dangerous? Nova Science, New York.

Busby, C., 2021. The Hiroshima A-bomb black rain and the lifespan study; a resolution of the enigma. Cancer Investig. 39, 902–907.

Busby, C., 2022. Ionizing radiation and cancer: the failure of the risk model. Cancer Treat. Res. Commun. 31, 100565.

Calabrese, E.J., 1999. Evidence that hormesis represents an "overcompensation" response to a disruption in homeostasis. Ecotox. Environ. Safety 42, 135–137.

Calabrese, E.J., 2005. Historical blunders: how toxicology got the dose-response relationship half right. Cell. Mol. Biol. (Noisy-le-grand) 51, 643–654.

Calabrese, E.J., 2008. Hormesis: why it is important to toxicology and toxicologists. Environ. Toxicol. Chem. 27, 1451–1474.

Calabrese, E.J., 2009. Getting the dose-response wrong: why hormesis became marginalized and the threshold model accepted. Arch. Toxicol. 83, 227–247.

Calabrese, E.J., 2010. Hormesis is central to toxicology, pharmacology and risk assessment. Hum. Exp. Toxicol. 29, 249–261.

Calabrese, E.J., 2011. Toxicology rewrites its history and rethinks its future: giving equal focus to both harmful and beneficial effects. Environ. Toxicol. Chem. 30, 2658–2673.

Calabrese, E.J., 2013. Hormetic mechanisms. Crit. Rev. Toxicol. 43, 580–606.

Calabrese, E.J., 2015. Hormesis: principles and applications. Homeopathy 104, 69–82.

Calabrese, E.J., 2016a. Preconditioning is hormesis part 1: documentation, dose-response features and mechanistic foundations. Pharm. Res. 110, 242–264.

Calabrese, E.J., 2016b. Preconditioning is hormesis part II: how the conditioning dose mediates protection: dose optimization within temporal and mechanistic frameworks. Pharm. Res. 110, 265–275.

Calabrese, E.J., 2017. Hormesis commonly observed in the assessment of aneuploidy in yeast. Environ. Pollut. 225, 713–728.

Calabrese, E.J., 2018. Hormesis: path and progression to significance. Int. J. Mol. Sci. 19, 2871.

Calabrese, E.J., Baldwin, L.A., 2000a. Chemical hormesis: its historical foundations as a biological hypothesis. Hum. Exp. Toxicol. 19, 2–31.

Calabrese, E.J., Baldwin, L.A., 2000b. Radiation hormesis: its historical foundations as a biological hypothesis. Hum. Exp. Toxicol. 19, 41–75.

Calabrese, E.J., Baldwin, L.A., 2000c. Radiation hormesis: the demise of a legitimate hypothesis. Hum. Exp. Toxicol. 19, 76–84.

Calabrese, E.J., Baldwin, L.A., 2000d. Tales of two similar hypotheses: the rise and fall of chemical and radiation hormesis. Hum. Exp. Toxicol. 19, 85–97.

Calabrese, E.J., Baldwin, L.A., 2000e. The marginalization of hormesis. Hum. Exp. Toxicol. 19, 32–40.

Calabrese, E.J., Baldwin, L.A., 2001. The frequency of U-shaped dose responses in the toxicological literature. Toxicol. Sci. 62, 330–338.

Calabrese, E.J., Baldwin, L.A., 2002. Defining hormesis. Hum. Exp. Toxicol. 21, 91–97.

Calabrese, E.J., Baldwin, L.A., 2003. The hormetic dose-response model is more common than the threshold model in toxicology. Toxicol. Sci. 71, 246–250.

Calabrese, E.J., Blain, R.B., 2005. The occurrence of hormetic dose responses in the toxicological literature, the hormesis database: an overview. Toxicol. Appl. Pharmacol. 202, 289–301.

Calabrese, E.J., Blain, R.B., 2011. The hormesis database: the occurrence of hormetic dose responses in the toxicological literature. Regul. Toxicol. Pharmacol. 61, 73–81.

Calabrese, E.J., Kozumbo, W.J., 2021. The hormetic dose-response mechanism: Nrfs activation. Pharmacol. Res. 167, 105526.

Calabrese, E.J., Mattson, M.P., 2011. Hormesis provides a generalized quantitative estimate of biological plasticity. J. Cell Comm. Signal. 5, 25–38.

Calabrese, E.J., O'Connor, M.K., 2014. Estimating risk of low radiation doses - a critical review of the BEIR VII report and its use of the linear no-threshold (LNT) hypothesis. Radiat. Res. 182, 463–474.

Calabrese, E.J., Staudenmayer, J.W., Stanek 3rd, E.J., Hoffmann, G.R., 2006. Hormesis outperforms threshold model in National Cancer Institute antitumor drug screening database. Toxicol. Sci. 94, 368–378.

Calabrese, E.J., Stanek 3rd, E.J., Nascarella, M.A., Hoffmann, G.R., 2008. Hormesis predicts low-dose responses better than threshold models. Int. J. Toxicol. 27, 369–378.

Calabrese, E.J., Agathokleous, E., Dhawan, G., Kapoor, R., Dhawan, V., Manes, P.K., Calabrese, V., 2023a. Nitric oxide and hormesis. Nitric Oxide 133, 1–17.

Calabrese, E.J., Pressman, P., Wallace Hayes, A., Dhawan, G., Kapoor, R., Calabrese, V., Agathokleous, E., Iavicoli, I., Giordano, J., 2023b. Hormesis, Biological Plasticity, and Implications for Clinical Trial Research (Submitted).

Cardarelli 2nd, J.J., Ulsh, B.A., 2018. It is time to move beyond the linear no-threshold theory for low-dose radiation protection. Dose-Response 16, 1559325818779651.

Cha, E.S., Zablotska, L.B., Bang, Y.J., Lee, W.J., 2020. Occupational radiation exposure and morbidity of circulatory disease among diagnostic medical radiation workers in South Korea. Occup. Environ. Med. 77, 752–760.

Chen, N., Wu, L., Yuan, H., Wang, J., 2015. ROS/autophagy/Nrf2 pathway mediated low-dose radiation induced radio-resistance in human lung adenocarcinoma A549 cell. Int. J. Biol. Sci. 11, 833–844.

Chu, S.H., Huang, M., Kelly, R.S., Benedetti, E., Siddiqui, J.K., Zeleznik, O.A., Pereira, A., Herrington, D., Wheelock, C.E., Krumsiek, J., McGeachie, M., Moore, S.C., Kraft, P., Mathé, E., Lasky-Su, J., Consortium of Metabolomics Studies Statistics Working Group, 2019. Integration of metabolomic and other omics data in population-based study designs: an epidemiological perspective. Metabolites. 9, 117.

Cioffi, D.L., Fontana, L., Leso, V., Dolce, P., Vitale, R., Vetrani, I., Galdi, A., Iavicoli, I., 2020. Low-dose ionizing radiation exposure and risk of thyroid functional alterations in healthcare workers. Eur. J. Radiol. 132, 109279.

Devic, C., Ferlazzo, M.L., Berthel, E., Foray, N., 2020. Influence of individual radiosensitivity on the hormesis phenomenon: toward a mechanistic explanation based on the Nucleoshuttling of ATM protein. Dose-Response 18, 1559325820913784.

Doss, M., 2013. Linear no-threshold model VS. radiation hormesis. Dose-Response 11, 480–497.

Durović, B., Spasić-Jokić, V., Durović, B., 2008. Influence of occupational exposure to low-dose ionizing radiation on the plasma activity of superoxide dismutase and glutathione level. Vojnosanit. Pregl. 65, 613–618.

Fornalski, K.W., Dobrzynski, L., 2009. Ionising radiation and the health of nuclear industry workers. Int. J. Low Radiat. 6, 57–78. https://doi.org/10.1504/IJLR.2009.026240.

Gillies, M., Kuznetsova, I., Sokolnikov, M., Haylock, R., O'Hagan, J., Tsareva, Y., Labutina, E., 2017. Lung cancer risk from plutonium: a pooled analysis of the Mayak and Sellafield worker cohorts. Radiat. Res. 188, 645–660.

Grellier, J., Atkinson, W., Bérard, P., Bingham, D., Birchall, A., Blanchardon, E., Bull, R., Guseva Canu, I., Challeton-de Vathaire, C., Cockerill, R., Do, M.T., Engels, H., Figuerola, J., Foster, A., Holmstock, L., Hurtgen, C., Laurier, D., Puncher, M., Riddell, A.E., Samson, E., Thierry-Chef, I., Tirmarche, M., Vrijheid, M., Cardis, E., 2017. Risk of lung Cancer mortality in nuclear workers from internal exposure to alpha particle-emitting radionuclides. Epidemiology 28, 675–684.

Gyuleva, I.M., Penkova, K.I., Rupova, I.T., Panova, D.Y., Djounova, J.N., 2015. Assessment of some immune parameters in occupationally exposed nuclear power plant workers: flow cytometry measurements of T lymphocyte subpopulations and immunoglobulin determination. Dose-Response 13, 1559325815611901.

Hajian, Tilaki K., 2012. Methodological issues of confounding in analytical epidemiologic studies. Caspian J. Intern. Med. 3, 488–495.

Hall, E.J., Brenner, D.J., 2008. Cancer risks from diagnostic radiology. Br. J. Radiol. 81, 362–378.

Hauptmann, M., Mohan, A.K., Doody, M.M., Linet, M.S., Mabuchi, K., 2003. Mortality from diseases of the circulatory system in radiologic technologists in the United States. Am. J. Epidemiol. 157, 239–248.
Iavicoli, I., Fontana, L., Leso, V., Calabrese, E.J., 2014. Hormetic dose-responses in nanotechnology studies. Sci. Total Environ. 487, 361–374.
Iavicoli, I., Leso, V., Fontana, L., Calabrese, E.J., 2018. Nanoparticle exposure and hormetic dose-responses: an update. Int. J. Mol. Sci. 19, 805.
Iavicoli, I., Fontana, L., Agathokleous, E., Santocono, C., Russo, F., Vetrani, I., Fedele, M., Calabrese, E.J., 2021. Hormetic dose responses induced by antibiotics in bacteria: a phantom menace to be thoroughly evaluated to address the environmental risk and tackle the antibiotic resistance phenomenon. Sci. Total Environ. 798, 149255.
ICPR (International Commission on Radiological Protection), 2007. The 2007 recommendations of the international commission on radiological protection. ICRP publication 103. Ann. ICRP 37, 1–332.
Iwasaki, T., Murata, M., Ohshima, S., Miyake, T., Kudo, S., Inoue, Y., Narita, M., Yoshimura, T., Akiba, S., Tango, T., Yoshimoto, Y., Shimizu, Y., Sobue, T., Kusumi, S., Yamagishi, C., Matsudaira, H., 2003. Second analysis of mortality of nuclear industry workers in Japan, 1986-1997. Radiat. Res. 159, 228–238.
Jargin, S.V., 2020. Radiation safety and Hormesis. Front. Public Health 8, 278.
Jeong, M., Jin, Y.W., Yang, K.H., Ahn, Y.O., Cha, C.Y., 2010. Radiation exposure and cancer incidence in a cohort of nuclear power industry workers in the Republic of Korea, 1992-2005. Radiat. Environ. Biophys. 49, 47–55.
Ji, K., Wang, Y., Du, L., Xu, C., Liu, Y., He, N., Wang, J., Liu, Q., 2019. Research progress on the biological effects of low-dose radiation in China. Dose-Response 17, 1559325819833488.
Kabilan, U., Graber, T.E., Alain, T., Klokov, D., 2020. Ionizing radiation and translation control: a link to radiation hormesis? Int. J. Mol. Sci. 21, 6650.
Linet, M.S., Kitahara, C.M., Ntowe, E., Kleinerman, R.A., Gilbert, E.S., Naito, N., Lipner, R.S., Miller, D.L., Berrington de González, A., Multi-Specialty Occupational Health Group, 2017. Mortality in U.S. physicians likely to perform fluoroscopy-guided interventional procedures compared with psychiatrists, 1979 to 2008. Radiology 284, 482–494.
Liu, J.J., Freedman, D.M., Little, M.P., Doody, M.M., Alexander, B.H., Kitahara, C.M., Lee, T., Rajaraman, P., Miller, J.S., Kampa, D.M., Simon, S.L., Preston, D.L., Linet, M. S., 2014. Work history and mortality risks in 90,268 US radiological technologists. Occup. Environ. Med. 71, 819–835.
López de Maturana, E., Pineda, S., Brand, A., Van Steen, K., Malats, N., 2016. Toward the integration of omics data in epidemiological studies: still a “long and winding road”. Genet. Epidemiol. 40, 558–569.
Luckey, T.D., 1980. Hormesis With Ionizing Radiation. CRC Press, Inc., Boca Raton, FL.
Luckey, T.D., 1991. Radiation Hormesis. CRC Press, Inc., Boca Raton, FL.
Luckey, T.D., Lawrence, K.S., 2006. Radiation hormesis: the good, the bad, and the ugly. Dose-Response 4, 169–190.
Matanoski, G.M., Sternberg, A., Elliott, E.A., 1987. Does radiation exposure produce a protective effect among radiologists? Health Phys. 52, 637–643.
Mattson, M.P., 2008. Hormesis defined. Ageing Res. Rev. 7, 1–7.
Meijers, J.M., Swaen, G.M., Schreiber, G.H., Sturmans, F., 1992. Occupational epidemiological studies in risk assessment and their relation to animal experimental data. Regul. Toxicol. Pharmacol. 16, 215–222.
Metz-Flamant, C., Samson, E., Caër-Lorho, S., Acker, A., Laurier, D., 2012. Leukemia risk associated with chronic external exposure to ionizing radiation in a French cohort of nuclear workers. Radiat. Res. 178, 489–498.
Mohan, A.K., Hauptmann, M., Freedman, D.M., Ron, E., Matanoski, G.M., Lubin, J.H., Alexander, B.H., Boice Jr., J.D., Doody, M.M., Linet, M.S., 2003. Cancer and other causes of mortality among radiologic technologists in the United States. Int. J. Cancer 103, 259–267.
Mothersill, C., Seymour, C., 2022. Radiation hormesis and dose response: are our current concepts meaningful or useful? Curr. Opin. Toxicol. 30, 100335.
Mundt, K.A., May, S., 2001. Epidemiological assessment of hormesis in studies with low-level exposure. Hum. Ecol. Risk. Assess. 7, 795–809.
NAS/NRC (National Academy of Sciences/National Research Council), 1956. The biological effects of atomic radiation: a report to the public. NAS/NRC, Washington DC.
NAS/NRC (National Academy of Sciences/National Research Council), 1960. The biological effects of atomic radiation: Summary reports. NAS/NRC, Washington DC.
NCRP (National Council on Radiation Protection and Measurements), 1995. Principles and Application of Collective Dose in Radiation Protection NCRP Report No. 121. National Council on Radiation Protection and Measurements, Washington DC.
NCRP (National Council on Radiation Protection and Measurements), 2015. Health Effects of Low Doses of Radiation: Perspectives on Integrating Radiation Biology and Epidemiology. Commentary No. 24. National Council on Radiation Protection and Measurements, Washington DC.
NCRP (National Council on Radiation Protection and Measurements), 2020. Approaches for Integrating Information from Radiation Biology and Epidemiology to Enhance Low-Dose Health Risk Assessment. Commentary No. 186. National Council on Radiation Protection and Measurements, Washington DC.
NIOSH (National Institute for Occupational Safety and Health), 2001. Mortality Patterns Among Uranium Enrichment Workers at the Portsmouth Gaseous Diffusion Plant Piketon, Ohio. Final Report. The Health-Related Energy Research Branch – Division of Surveillance, Hazard Evaluations, and Field Studies, National Institute for Occupational Safety and Health. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention.
NRC (National Research Council), 1983. Risk Assessment in the Federal Government: Managing the Process. Committee on the Institutional Means for Assessment of Risks to Public Health, Commission on Life Sciences. 1983. National Academy Press, Washington D.C. (ISBN: 0-309-03349-7).
NRC (National Research Council), 2006. Committee to Assess Health Risks From Exposure to Low Level of Ionizing Radiation. Health Risks From Exposure to Low Levels of Ionizing Radiation: BEIR VII Phase 2. National Research Council. National Academies Press (US), Washington (DC).
NRC (National Research Council), 2012. Committee on the Analysis of Cancer Risks in Populations near Nuclear Facilities-Phase I; Nuclear and Radiation Studies Board; Division on Earth and Life Studies; National Research Council. National Academies Press (US), Washington (DC).
Paraswani, N., Thoh, M., Bhilwade, H.N., Ghosh, A., 2018. Early antioxidant responses via the concerted activation of NF-κB and Nrf2 characterize the gamma-radiation-induced adaptive response in quiescent human peripheral blood mononuclear cells. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 831, 50–61.
Parsons, P.A., 1990. Radiation hormesis: an evolutionary expectation and the evidence. Int. J. Rad. Appl. Instrum. A 41, 857–860.
Pennington, C.W., Siegel, J.A., 2019. The linear no-threshold model of low-dose radiogenic cancer: a failed fiction. Dose-Response 17, 1559325818824200.
Pierce, D.A., Sharp, G.B., Mabuchi, K., 2005. Joint effects of radiation and smoking on lung cancer risk among atomic bomb survivors. Radiat. Res. 163, 694–695.
Pinkerton, L.E., Waters, M.A., Hein, M.J., Zivkovich, Z., Schubauer-Berigan, M.K., Grajewski, B., 2012. Cause-specific mortality among a cohort of U.S. flight attendants. Am. J. Ind. Med. 55, 25–36.
Pollycove, M., Feinendegen, L.E., 2001. Biologic responses to low doses of ionizing radiation: detriment versus hormesis. Part 2. Dose responses of organisms. J. Nucl. Med. 42, 26N–37N.
Preston, D.L., Kitahara, C.M., Freedman, D.M., Sigurdson, A.J., Simon, S.L., Little, M.P., Cahoon, E.K., Rajaraman, P., Miller, J.S., Alexander, B.H., Doody, M.M., Linet, M.S., 2016. Breast cancer risk and protracted low-to-moderate dose occupational radiation exposure in the US Radiologic Technologists Cohort, 1983-2008. Br. J. Cancer 115, 1105–1112.
Russo, G.L., Tedesco, I., Russo, M., Cioppa, A., Andreassi, M.G., Picano, E., 2012. Cellular adaptive response to chronic radiation exposure in interventional cardiologists. Eur. Heart J. 33, 408–414.
Sacks, B., Meyerson, G., Siegel, J.A., 2016. Epidemiology without biology: false paradigms, unfounded assumptions, and specious statistics in radiation science (with commentaries by Inge Schmitz-Feuerhake and Christopher Busby and a reply by the authors). Biol. Theory 11, 69–101.
Sagan, L.A., 1987. What is hormesis and why haven’t we heard about it before? Health Phys. 52, 521–525.
Schulz, H., 1887. Zur Lehre von der Arzneiwirdung. Arch. F. Pathol. Anat. 108, 423–445.
Schulz, H., 1888. Ueber Hefegifte. Pflugers Arch. 42, 517–541.
Scott, B.R., 2018. A critique of recent epidemiologic studies of Cancer mortality among nuclear workers. Dose-Response 16, 1559325818778702.
Scott, B.R., 2021. Some epidemiologic studies of low-dose-radiation cancer risks are misinforming. Dose-Response 19, 15593258211024499.
Seong, K.M., Seo, S., Lee, D., Kim, M.J., Lee, S.S., Park, S., Jin, Y.W., 2016. Is the linear no-threshold dose-response paradigm still necessary for the assessment of health effects of low-dose radiation? J. Korean Med. Sci. 31 (Suppl. 1), S10–S23.
Shibamoto, Y., Nakamura, H., 2018. Overview of biological, epidemiological, and clinical evidence of radiation hormesis. Int. J. Mol. Sci. 19, 2387.
Shuryak, I., Loucas, B.D., Cornforth, M.N., 2021. Robbing Peter to pay Paul: competition for radiogenic breaks during rejoining diminishes curvature in the dose response for simple chromosome exchanges. Radiat. Res. 196, 147–155.
Smith, M.T., Hainaut, P., Perera, F., Schulte, P.A., Boffetta, P., Chanock, S.J., Rothman, N., 2011. Future perspectives on molecular epidemiology. IARC Sci. Publ. 163, 493–500.
Southam, C.M., Ehrlich, J., 1943. Effects of extracts of western red-cedar heartwood on certain wood-decaying fungi in culture. Phytopathology 33, 517–524.
Sponsler, R., Cameron, J.R., 2005. Nuclear Shipyard Worker Study (1980–1988): a large cohort exposed to low-dose-rate gamma radiation. Int. J. Low Radiat. 1, 463–478.
Subedi, P., Moertl, S., Azimzadeh, O., 2022. Omics in radiation biology: surprised but not disappointed. Radiation 2, 124–129.
Sutou, S., 2022. Low dose radiation effects. Curr. Opin. Toxicol. 30, 100329.
Tian, X.L., Lu, X., Lyu, Y.M., Zhao, H., Liu, Q.J., Tian, M., 2022. Analysis of red blood cells and their components in medical workers with occupational exposure to low-dose ionizing radiation. Dose-Response 20, 15593258221081373.
Tokarskaya, Z.B., Okladnikova, N.D., Belyaeva, Z.D., Drozhko, E.G., 1997. Multifactorial analysis of lung cancer dose-response relationships for workers at the Mayak nuclear enterprise. Health Phys. 73, 899–905.
de Toledo, S.M., Asaad, N., Venkatachalam, P., Li, L., Howell, R.W., Spitz, D.R., Azzam, E.I., 2006. Adaptive responses to low-dose/low-dose-rate gamma rays in normal human fibroblasts: the role of growth architecture and oxidative metabolism. Radiat. Res. 166, 849–857.
Tubiana, M., 2005. Dose-effect relationship and estimation of the carcinogenic effects of low doses of ionizing radiation: the joint report of the Académie des Sciences (Paris) and of the Académie Nationale de Médecine. Int. J. Radiat. Oncol. Biol. Phys. 63, 317–319.
UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation), 2012. Biological Mechanisms of Radiation Actions at Low Doses: A White Paper to Guide the Scientific Committee’s Future Programme of Work. United Nations, New York, NY.
Vaiserman, A.M., 2010. Radiation hormesis: historical perspective and implications for low-dose cancer risk assessment. Dose-Response 8, 172–191.
Vaiserman, A., Koliada, A., Zabuga, O., Socol, Y., 2018. Health impacts of low dose ionizing radiation: current scientific debates and regulatory issues. Dose-Response 16, 1559325818796331.

Valentin, J., 2005. Low-dose extrapolation of radiation-related cancer risk. Ann. ICRP 35, 1–140.
Wakeford, R., 2009. Radiation in the workplace-a review of studies of the risks of occupational exposure to ionising radiation. J. Radiol. Prot. 29 (2A), A61–A79.
Wu, S., Zhu, W., Thompson, P., Hannun, Y.A., 2018. Evaluating intrinsic and non-intrinsic cancer risk factors. Nat. Commun. 9, 3490.
Yamaoka, K., 2006. Activation of antioxidant system by low dose radiation and its applicable possibility for treatment of reactive oxygen species-related diseases. J. Clin. Biochem. Nutr. 39, 114–133.
Zablotska, L.B., Ashmore, J.P., Howe, G.R., 2004. Analysis of mortality among Canadian nuclear power industry workers after chronic low-dose exposure to ionizing radiation. Radiat. Res. 161, 633–641.
Zeeb, H., Blettner, M., Hammer, G.P., Langner, I., 2002. Cohort mortality study of German cockpit crew, 1960-1997. Epidemiology 13, 693–699.