00022 Improved Approaches to Dose–Response Modeling of Toxicological and Adaptive Endpoints for Risk Assessment: Hormetic Dose Response

Edward J. Calabrese, School of Public Health and Health Sciences, Department of Environmental Health Sciences, University of Massachusetts, Amherst, MA, United States

Abstract

This article provides a general description and evaluation of the hormetic biphasic dose–response relationship, including its historical foundation, how it became marginalized in the early part of the 20th century, and why it has experienced a strong resurgence in interest and application over the past several decades. The article provides scientific documentation concerning the occurrence, frequency, generality, and mechanistic basis for hormetic dose responses. Hormetic effects occur without restriction to biological model, cell type, endpoint measured, inducing agent, and level of biological organization (cell, organ, and organism). The quantitative features of hormetic dose responses are also independent of mechanism. The hormetic dose response has also outcompeted both the threshold and linear nonthreshold models in making accurate predictions in the low-dose zone using multiple large datasets with a wide range of models, agents, and endpoints. Particular attention is also given to how risk assessment methods can integrate the hormetic concept for carcinogens and noncarcinogens, improve the accuracy of low-dose risk estimates, and enhance public health outcomes. The hormetic dose response is therefore central to biology, toxicology, pharmacology, and public health and needs to be incorporated into governmental risk assessment procedures.

Introduction

The dose–response relationship is central to toxicology, risk assessment, and public health. Since the onset of the so-called modern era of toxicology, such as following the National Environmental Protection Act (NEPA) legislation in 1969 in the United States that led to the creation of EPA and OSHA in 1970, life has not been the same for the fields of toxicology and risk assessment. During the 1970s and thereafter, there has been a plethora of federal legislative acts that addressed pollution concerns with respect to streams, rivers and coastal environments, drinking water, air, agents with toxic potential entering commerce, the disposal of hazardous wastes, and numerous statutes reducing the exposure to lead, among others. These actions were often linked to activities of the US National Academy of Sciences (NAS) to provide guidance to various federal agencies on matters related to risk assessment. Regardless of the legislation, statute, or guidance document offered by the NAS on these specific matters, each document was ultimately based on understandings and assumptions of the nature of the dose response in the low-dose zone. That is, the dose–response concept was front and center, guiding risk assessment and public health activities at the national, state, and local levels. Thus, it was critical that the NAS and the regulatory and public health agencies got the principles, adequate supportive scientific/experimental documentation, and applications of the dose–response concept correct since failure to do so could have profound effects on the public health, the economy, and the national and international well-being.

Since the creation of EPA, a major issue has been how to estimate risk from agents that could induce cancer and those that were most likely not carcinogens. In fact, the resolution of this issue would come to dominate a substantial portion of the environmental toxicology and risk assessment landscape during the first decade of EPA's and OSHA's existence. The real conundrum for the regulatory agencies was how to deal with the carcinogen issue. That is, should carcinogens be treated differently in the risk assessment process than agents that did not cause cancer? Realizing that governmental processes can often move slowly, it took over a decade for an apparent consensus resolution for carcinogen risk assessment to emerge. Such actions would be highlighted by a massive effort by OSHA to conduct a long-standing 2-year public hearing/rule making period during the last years of the 1970s to assist in deriving a carcinogen assessment policy. This 250,000-page record resulted in the publication in 1980 of the OSHA generic cancer risk assessment policy (**OSHA, 1980**).

This brief excursion down the risk assessment memory lane is valuable as it helps to establish the context within which modern risk assessment evolved. However, despite the fact that modern risk assessment seems to have been born around 1970 with the start of the legislative environmental revolution, apparently kicked off some 8 years earlier with the publication of *Silent Spring* by Rachael Carson in the fall of 1962, the dose–response concept had a rich prior history stretching back nearly a century, which provided the foundation for actions on dose response and risk assessment by advisory bodies such as the NAS and the various federal and state regulatory agencies.

The Birth of Dose–Response Modernity

While debates have long persisted over the validity and use of the threshold and linear non-threshold (LNT) dose–response relationships, the first research-based insights into the nature of the dose response emerged from domain of microbiology, centering on the landmark works of Joseph Lister concerning aseptic surgery and the findings of Koch about how to efficiently kill anthrax spores. It is generally recognized that **Koch (1881)** initiated the process of assessing the bactericidal effects of numerous chemical disinfectants via the use of pure cultures of bacteria. His initial approach was to assess the survival of emulsions of anthrax spores that were dried upon silk threads. He would determine the time of spore survival in solutions of the then known disinfectants. The approach of Koch would be progressively modified resulting in more reliable microbiological methods (**Chick, 1908**). It was therefore Koch who published the first systematic findings on the killing of anthrax spores with the then popular biocide, carbolic acid, which was first used by Lister.

While Koch started the research on disinfectant potency and dose response, he inspired the actions of many others. One particular group led by **Kronig and Paul (1897)** provided an assessment of disinfectant properties via the use of well-defined microorganisms, the use of numerous disinfectants, and all within the framework of a broad range of concentrations. Their efforts revealed a logarithmic relationship between the numbers of bacteria surviving the chemical disinfection treatment and survival times, an observation confirmed by others (**Chick, 1908; Madsen and Nyman, 1907**). Of importance to the dose–response debate was that **Chick (1908)** noted that the dose–time–response was very similar to a first-order reaction that she called a unimolecular reaction. Of further significance was that this description is based on the law of mass action wherein the velocity of the reaction is proportional to the active mass of reacting agent present at that time. In effect, Chick would claim that the velocity of disinfection at any instant is proportional to the number (or weight) of living bacteria present.

The thinking of Chick was important not only for its application to disinfection practices at the community level but also because of its capacity to be generalized to a broad range of agents having environmental relevance, including the then emerging field of radiation biology. For example, **Blau and Altenburger (1922)**, expanding the perspective of Chick, found that the destruction of microorganisms by X-rays resulted in unimolecular dose–response curves. Based upon such observations, these authors concluded that the death of cells was mediated via one or at most a few quanta of energy. Such efforts provided the experimental and intellectual framework that was adopted by leading physicists for radiation target theory (**Crowther, 1926; Holweek and Lacassagne, 1930; Pugsley et al., 1935; Wyckoff, 1930**), which was subsequently applied to the newly emerging X-ray mutational data of **Muller (1928)** and **Timoféeff-Ressovsky et al. (1935)** leading to the LNT single-hit theory for genomic mutations, an hypothesis later generalized for radiation and chemically induced cancers (**Calabrese, 2015**). Thus, the theoretical basis of radiation dose response and the LNT single-hit theory evolved directly from the work of Chick on chemical disinfection. As noted by **Packard (1931)** and **Clark (1937)**, the radiation and chemical dose–response controversies are similar and depend upon the need to resolve similar biological/toxicological issues.

The unimolecular hypothesis of Chick created considerable debate in the microbiological literature for the next several decades as seen initially in the reports of **Arrhenius (1915)**; **Eijkman (1908)**; **Hewlett (1909)**; and **Reichel (1909)**. Such reports were followed by those of **Loeb and Northrop (1917)**; **Brooks (1918)**; **Peters (1920)**; **Smith (1921,1923)**; **Shackell (1925)**, and **Shackell et al. (1924/1925)**. These discussions and criticisms eventually coalesced in the writings of **Buchanan and Fulmer (1930)** who noted (a) a lack of correspondence of the experimental evidence with the theoretical curve at the beginning of the experiment. They were critical of the Chick findings since such studies had inadequate data at short intervals at the temporal start of the experiments. The critics concluded that Chick's findings simply could not provide a proper assessment of the distribution of susceptibility in the population of bacteria. This was a serious criticism since it undercut predictions at the low end of the distribution. (b)There was also a lack of association of the actual survivors' curve with the logarithmic curve in the later part of the response curve. For example, when the values of the velocity constant are small, they tend to decrease rather than remain constant as predicted. (c) There were also scientific issues with the assumption of uniform susceptibility. Substantial data indicate considerable variability in susceptibility. Furthermore, the ratio of susceptible to non-susceptible cell is not constant, which should be the case if the unimolecular model was correct. (d) There were also problems related to providing a theoretical basis for interpreting cell death via a unimolecular reaction.

These criticisms would lead to a general rejection of the unimolecular dose–response concept within most areas of the biological sciences, with the major exception of radiation biology and with its particular focus of the induction of mutations by ionizing radiation. The unimolecular dose–response model was therefore widely outcompeted by the characteristic dose–response theory. This dose–response theory would be built upon an estimation of the distribution of individual variation within the population with regard to susceptibility to toxic substances, including chemical and physical agents.

The most important and penetrating development of the characteristic dose response was published by **Trevan (1927)**. This work was based on a strong dissatisfaction with predictions such as those derived from the unimolecular approach to estimate minimal effective or toxic doses. He argued that this approach should be replaced with a method that estimates the central tendency of the group response. This concept led Trevan to derive the concept and terminology of the lethal dose 50 (**Trevan, 1927**). He also created the term "characteristic" to describe the dose response representing the percentage of response (e.g., mortality or other biological responses of interest or concern) induced by a range of doses of a drug, industrial agent, or radiation on biological models. Trevan then provided the statistical basis for the key practical issues of sample size and statistical power. A strength of the Trevan proposal was that it could provide a statistical vehicle to simulate a response descriptor that could be reliably determined, thereby offering a powerful incentive for additional research.

The leadership of Trevan was extended by collective efforts of Bliss and Gaddum who would integrate the concepts of Trevan into the major biological disciplines. For example, Bliss published a "how to" statistical road map for dose–response assessment for many key biological disciplines of the 1930–1960s, including entomology, microbiology, physiology, pharmacology, and toxicology (**Bliss, 1935a,b,c, Bliss, 1939, 1940, 1941, 1956, 1957; Bliss and Cattell, 1943; Bliss and Packard, 1941**). The writings of Bliss and Gaddum (**Gaddum, 1945, 1953**) became the standard and would be used as basic instructional tools in the education and training of generations of biological researchers and, in many respects, remain so today.

The impact of these biostatistical leaders of the earlier/mid decades of the 20th century was profound. They also provided the foundation for follow-up work by **Finney (1943/1944)**, **Finney (1949, 1952)** on the development of probit analysis, with its important applications to the field of toxicology. In fact, it can be seen that such thinking was having a significant impact on the then nascent field of cancer risk assessment with its applications in the classic paper of **Bryan and Shimkin (1940)**. This paper evaluated the nature of the dose–response relationship for various chemical carcinogens over a wide range of exposures. It is also ironic that the authors of this paper constrained the dose–response model to estimate only tumor increases, thereby obscuring apparent hormetic effects assuming such responses reflect variability rather than representative treatment effects. Two decades later, the probit analysis approach of Finney would

provide the foundation of the influential Mantel–Bryan model (**Mantel and Bryan, 1961**) for low-dose cancer risk assessment as is typically applied when extrapolating far beyond the observable empirical data to very low risks in the one in a million and lower zones for lifetime risks.

In setting the context of the history, documentation, and applications of dose responses in the 20th century, we therefore find that two theories dominated the mainstream scientific community era. These were the unimolecular and the characteristic models. While the unimolecular model was initially more powerful in its appeal and applications, it would be challenged seriously by the characteristic model advocates and lose influence. There were also attempts to broaden and reformulate the characteristic dose–response concept as one describing biochemical process that mediated interindividual variation. Moreover, this transformation acquired multiple mathematical forms even down to the present time as seen in various hit and stage theories of carcinogenesis.

Other biostatistical approaches were also developed, such as the logistic method (**Berkson, 1944, 1951; Reed and Berkson, 1929**). For example, the use of logits with quantal data was founded on the assumption that the logarithms of the individual doses were distributed in a complex curve slightly different than that upon which the probit model was based. In fact, **Emmens (1940/1941)** attempted to account for the dose–response curve for mortality as an example using the logistic approach. He further argued that if the concept of tolerance was abandoned due to theoretical assumptions, then the law of chance would favor the use of logits. This view was formulated earlier by **Yule (1910)** in a more fundamental manner, using a random-hit theory dose–response method, with the dose response offering similar features to that seen with the probit curve method.

The issue of dose–response acceptance was important as it became an object of considerable focus in the influential text by Alfred J. Clark entitled *Handbook of Experimental Pharmacology* in 1937 (**Clark, 1937**). This text was critical of the unimolecular theory while providing support for the characteristic curve model, including detailed explanations concerning how it could be integrated into new developments reported with pharmacokinetic processes. However, even in Clark's extensive criticism of the unimolecular dose–response model, he was very respectful as seen in the comment that "it is obvious that a physicochemical theory (i.e., unimolecular theory) regarding the mode of action of drugs, which has received the support of Arrhenius must be considered carefully." The same type of respectful deference was not shown to Schulz and his biphasic dose response (to be discussed immediately in the succeeding text), rather, just the opposite. Of course, Arrhenius was a Nobel Prize recipient and chair of the Nobel Prize awarding committee.

The Forgotten Dose–Response Model: Biphasic Dose Response

We can thus see that dose–response debate and controversy did not start with the onset of the environmental revolution of the 1970s and the issues over how to estimate the risk of carcinogens at very low doses. In fact, the aforementioned discussion demonstrates that two groups of mainstream biological/biomedical scientists had explored and debated these issues for the previous half-century prior to the so-called modern dose–response era. Of particular relevance to the present paper is that it was within this dynamic intellectual environment that the issue of the hormetic biphasic dose response emerged and evolved. However, one thing is obvious right from the start. That is, unimolecular and the characteristic dose–response concepts originated within two opposing camps of mainstream scientists, and as a result, their conflicts would be followed, debated, and respected. However, what will become the hormetic dose response originated in an entirely different manner, emerging from the long-standing dispute between traditional medicine and homeopathy. Since the hormetic dose response was claimed by its discoverer, Hugo Schulz, to provide the explanatory principle of homeopathy, Schulz's biphasic dose–response model and he himself became the object of much criticism of both dose–response camps but especially of the emerging winning group of the early-mid-20th century, the characteristic curve model group, as highlighted in the very influential writings of Clark.

Despite its characterization as the forgotten dose response, the biphasic dose–response relationship was the first dose–response model to be experimentally formulated. The initial data underlying this development were generated by Hugo Schulz (1853–1932), a physician who was extremely well trained in pharmacology and toxicology. This research was undertaken at the University of Greifswald in northern Germany probably in late 1883, with his first presentation on this topic to the scientific community occurring at a local meeting of Greifswald Medical Society in 1884. Schulz had done extensive laboratory research assessing the effects of various chemical disinfectants on the survival and metabolism of yeasts (**Bohme, 1986**). In fact, he was a young contemporary of Koch who was doing similar research but with bacteria. Koch would soon become famous for his discoveries relating to the life cycle of anthrax. Koch would go on to create a powerful research program in basic and public health microbiology, with three of the first seven Nobel Prize winners in biology and medicine being from Koch's laboratory, including himself.

The scientific path of Schulz would be different. In his studies on the effects of the multiple chemical disinfectants, Schulz incorporated a broad dose–response feature, a time component, and a metabolic measure along with the standard mortality endpoint used by others. In fact, Schulz's study designs were more sophisticated and more robust than the future Nobel Prize winner Koch. As a result, Schulz observed an unexpected biphasic dose response in which high doses were toxic and suppressed metabolism, while the opposite seemed to occur at low doses. This troubled Schulz, making him think that he must have had some type of methodological error in his experiments. However, copious replications and other assessments gave him high confidence that his findings were real and reproducible.

These findings should have been of considerable interest to Robert Koch and Joseph Lister, among others. However, something happened at the next step of hypothesis development that changed the course of Schulz's professional life and the development of dose–response theory and practice down to the present time. The biphasic dose–response observations soon became integrated into a general biologically based dose–response framework by Schulz and his colleague at Greifswald, Rudolph Arndt. So convinced of the correctness and generality of their conceptual dose–response model, the creators designated their model a biological law, called Arndt–Schulz law. A pro-

tégé of Robert Koch, Ferdinand Hueppe, generalized their findings to bacteria, strangely renaming the phenomenon Hueppe's rule while at the same time acknowledging the primacy of Schulz (**Hueppe, 1896**).

In retrospect, this dose–response theory of Schulz and Arndt was conceptual and mostly intuitive, with the data supporting it limited but acceptable on its own merits. However, it was the integration across diverse studies and the interpretation of the data that was problematic. More specifically, Schulz was interested both in chemical disinfection and in testing features of homeopathy. With respect to the latter, Schulz learned of an 1884 study in which the homeopathic preparation called veratrine was used to successfully treat gastroenteritis in humans (**Bloedau, 1884**). This intrigued Schulz who went to Koch to obtain a pure culture of the bacterium causing the disease. Schulz wanted to test whether the veratrine could actually kill the causative agent and thereby obtain insight on the possible mechanism of the homeopathic treatment. However, regardless of the dose, the veratrine was unable to kill this disease-causing agent. While some scientists may have questioned the reliability of veratrine findings, Schulz and Arndt did not. In light of Schulz's other research with yeast, Schulz and Arndt came to the view that veratrine was an effective agent against gastroenteritis but it did so not by killing the bacteria itself, but by enhancing the adaptive capacity of the human to fight off the infection. They came to this conclusion by linking the yeast findings, which indicated that the large number of chemical disinfectants tested acted differently at low dose, enhancing survival. Thus, Arndt and Schulz developed the hypothesis that most agents act biphasically and that they induce adaptive survival-enhancing responses at low doses. They then applied this concept not only to the veratrine but also to homeopathic drugs in general. It was within this context that they derived the perspective that they had discovered the underlying explanatory principle of homeopathy. It was with the public announcement of this theory that the problems of Schulz and this biphasic dose–response model and eventually the term hormesis would begin.

The problem for Schulz and his model was that homeopathy and traditional medicine were in a major and long-standing conflict over which medical practice would come to dominate society (**Coulter, 1972, 1982**). There was much animosity over the issue. By linking his biphasic dose–response theory to homeopathy, Schulz ensured that it would become the object of profound criticism and would be rejected by the biomedical community. This should not have been hard to predict.

The biomedical community would go to great lengths to marginalize Schulz and his dose–response model. This started right away as evident in the contemporary literature and from multiple perspectives. The contemporary research rival Hueppe argued that the findings of Schulz should not be rejected even though he made the profound error of associating it with homeopathy (**Hueppe, 1896**). However, most critics were not so sympathetic. This may be best seen in the copious writings of Clark, who became a leading critic. Clark did his best to link Schulz with the high-dilution Hahnemann wing of homeopathy (see **Calabrese, 2005**; **Tables 1–3** for numerous examples of such efforts by Clark). This was done to both discredit Schulz and his dose response even though Schulz was adamant in his writings that he did not support the high-dilution views of followers of Hahnemann who argued that biological effects could occur below Avogadro's number (**Bohme, 1986**). For example, Clark would write that the Arndt–Schulz dose–response law was "in accord with homeopathic doctrines," implying that it derived its foundation from a homeopathic rather than a biological/toxicological tradition. Clark would also state that the Arndt–Schulz law "is obviously untrue in the case of most drugs that have been studied carefully" yet failing to provide the documentation to support such a conclusion.

The statements of Clark were also inconsistent with a substantial series of independent reports in the biological literature that were strongly supportive of the Schulz dose–response model (**Calabrese and Baldwin, 2000a,b,c,d,e**). However, the views of Clark would carry the day as Clark and many of his colleagues in British pharmacological community were prominent leaders in the domain of traditional medicine and extremely accomplished researchers in their own right. When matched against such a profoundly accomplished and committed opposition, Schulz would have little chance to influence to direction of the field. Furthermore, Schulz's career was so affected that he was unable to consider moving to more prestigious academic institutions, as was commonly done during that era, being relegated to Greifswald for his entire professional life. The travails of Schulz and his biphasic dose response were highlighted in a sympathetic memorializing of his life by a colleague, who recounted the challenges and unfair and often deceitful characterizations by otherwise leading scientists in that era, all in an effort to destroy homeopathy, making Schulz and the hormesis concept what today one might call collateral damage (**Wels, 1933**).

Despite those profound difficulties that Schulz endured, many researchers published findings of biphasic dose–response relationships, especially in the area plants, microbiology, and entomology with both chemicals and radiation. The findings of Schulz stimulated numerous doctoral dissertations (e.g., **Gottbrecht, 1886**; **Hoffman, 1884, 1922**; **Niethammer, 1927**; **Thol, 1885**) that generally confirmed and extended his findings. Numerous other dissertations addressing the stimulation of bacterial growth by low doses of toxic agents were conducted under the direction of Charles Winslow, the Yale University professor of bacteriology and longtime editor in chief of the *Journal of Bacteriology* and later the *American Journal of Public Health*. For example, **Hotchkiss (1923a,b)** provided a comprehensive survey of the stimulatory and inhibitory/toxic effects of both minerals and toxic metals on *E. coli*. Of particular interest was that the agents were usually tested over a broad concentration range with six or more doses. Most of the agents tested displayed a low-dose stimulation, including the salts of lead, mercury, nickel, tin, titanium, and strontium. The work of Hotchkiss revealed that the stimulatory response was strongly influenced by the nature and the quality of the study design. Experiments with large numbers of doses, especially with multiple treatments below the toxic threshold, displayed consistent stimulatory responses. The median maximum stimulatory responses were modest, being about 50% greater than the controls, while the stimulatory range was more variable, extending from 2- to 100-fold below the threshold, with an average of about 50-fold. The work of Hotchkiss was to stimulate a long line of subsequent graduate students at Yale University to extend these findings. Furthermore, the study design features implemented by Hotchkiss under the direction of Winslow created a type of research standard for the assessment hormetic-like biphasic dose responses in terms of number of doses, dose range and spacing, and replications. This research was significant as it led to the general recognition by the 1930s that disinfectants

Table 1 The first use of the term hormesis, Charles Southam's undergraduate thesis.

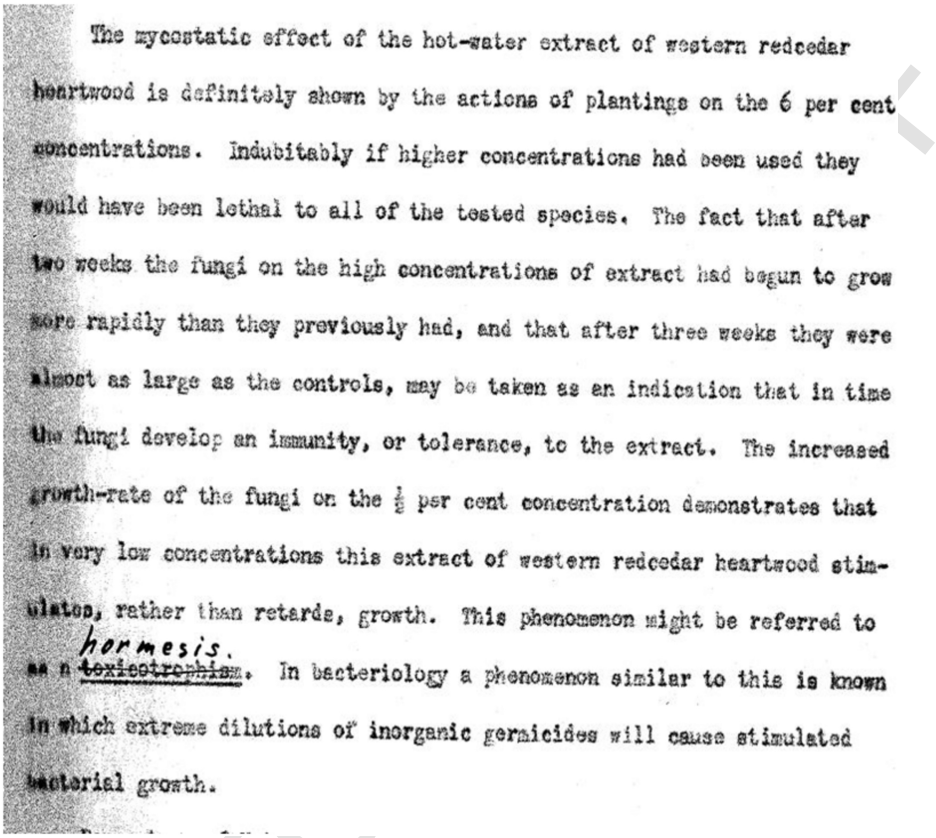
References	*Quote*
Southam (1941), p. 21	 The mycostatic effect of the hot-water extract of western redcedar heartwood is definitely shown by the actions of plantings on the 6 per cent concentrations. Indubitably if higher concentrations had been used they would have been lethal to all of the tested species. The fact that after two weeks the fungi on the high concentrations of extract had begun to grow more rapidly than they previously had, and that after three weeks they were almost as large as the controls, may be taken as an indication that in time the fungi develop an immunity, or tolerance, to the extract. The increased growth-rate of the fungi on the ½ per cent concentration demonstrates that in very low concentrations this extract of western redcedar heartwood stimulates, rather than retards, growth. This phenomenon might be referred to as a hormesis. ~~toxicotrophism~~. In bacteriology a phenomenon similar to this is known in which extreme dilutions of inorganic germicides will cause stimulated bacterial growth.

Table 2 Hormetic principles.

Low/modest stress induces pro-survival responses within well-defined temporal parameters
Hormetic effects may have inherent health benefits on their own or in preventing damage from a subsequent more harmful exposure (i.e., preconditioning) or when administered after the harmful exposure (i.e., postconditioning)
The quantitative features of the hormetic dose response are very similar across species and individuals and independent of differential susceptibility, agent potency, and mechanism
The amplitude of the stimulatory response is constrained by and defines the limits of plasticity within biological systems
Hormetic responses occur at all levels of biological organization, such as the cell, organ, individual, and population
Downstream processes from multiple independent stressor agents/excitatory stimuli yield an integrated dose response (i.e., molecular vector) reflecting the hormetic dose response
Hormesis may be either a general response to environmentally induced stress/damage or constrained by elements of chemical structure specificity for endpoint induction

Adapted from Calabrese, E.J., 2008a. Hormesis: Why it is important to toxicology and toxicologists. Environmental Toxicology and Chemistry 27, 1451–1474.

display a biphasic dose response, with knowledge of this phenomenon becoming so recognized and accepted that it became incorporated into standard microbiological texts during the middle decades of the 20th century (**Clifton, 1957; Lamanna and Mallette, 1965; Salle, 1939**).

The biphasic effects of disinfectants on bacteria were paralleled with similar findings concerning the effects of various toxic inorganic agents on the ammonification, nitrification, and nitrogen fixation in soil by various bacterial species. This research was initially studied in 1913 by the well-known bacteriologist Lipman (**Lipman and Wilson, 1913**) from the University of California at Berkeley who was in-

Table 3 Significant hormetic dose–response information.

Most commonly observed dose–response relationship using a priori entry and evaluative criteria
Distinct quantitative features, making it a unique biphasic dose–response relationship
Most significant feature is the modest amplitude of the stimulatory response, typically less than twice the control values
The low-dose stimulation may occur by a direct stimulation or an overcompensation to a disruption of homeostasis, which can range widely in magnitude of damage
Hormesis is an adaptive response that enhances tissue repair and protects against damage from subsequent and more massive exposures
Hormetic dose responses are very generalizable, being independent of biological model, endpoint measured, and chemical class
Numerous specific proximate mechanisms account for hormetic dose responses
Hormetic effects may occur very early in developmental processes but can lose efficiency during the aging process, especially in the elderly

Adapted from Calabrese, E.J., 2008a. Hormesis: Why it is important to toxicology and toxicologists. Environmental Toxicology and Chemistry 27, 1451–1474.

terested in assessing the impact on vast land areas of large quantities of waste alkali on the capacity of soil bacteria to perform ammonification and nitrification. The low-dose stimulation responses by bacterial ammonifiers were commonly observed. At the same time, **Greaves (1913a,b)** and **Greaves and Carter (1924)** revealed that various chemical insecticides likewise induced hormetic-like biphasic dose responses on the bacterial ammonification process. Greaves was unusual in his study designs, using from 20 to 30 concentrations over a wide concentration range. The findings of Greaves were noted for their consistency of responses between replicate studies. Similar findings were also reported for various uranium compounds, again with strong study designs (**Stoklasa and Penkava, 1928**).

The story of hormetic-like biphasic dose responses just briefly summarized for bacteria also occurred with fungi, yeast, insects, and plants using various chemicals and radiation as inducing agents during the early decades of the 20th century. The findings were often reported by experienced investigators, typically with adequate to strong study designs and published in the leading journals of that era. However, these findings were never adequately summarized and integrated during the 20th century. It was only during the resurgence of the hormesis concept at the very end of the 20th century that this extensive published network of early historical findings on hormetic dose responses was revealed. Of further note was that a German language journal *Cell Stimulation* was published during the 1920s. Likewise, an academic journal-like publication called the *Stimulation Newsletter* was published that addressed the capacity of radiation to induce stimulation in plant growth. The history of these activities has been reconstructed and published in an entire issue of the journal *Human and Experimental Toxicology* (**Calabrese and Baldwin, 2000a,b,c,d,e**).

These findings were to force some investigators to struggle with the defining of what the hormetic dose response was. Perhaps, the most significant theoretical debate centered on whether the low-dose stimulation was a direct one or an overcompensation to a disruption in homeostasis, that is, some minor degree of toxicity. A number of extremely well-designed and well-conducted studies with different biological models and inducing agents provided convincing evidence that a low-dose stimulation may occur as a result of an overcompensation to an induced initial toxicity. Of particular note were findings of **Branham (1929)** of the University of Rochester who sought to provide a very explicit, detailed, and advanced replication of the original findings of Schulz that stimulated interest in the biphasic dose–response concept. Her findings were striking in that she not only reported that low concentrations of numerous chemical disinfectants stimulated the growth of yeast colonies but also did so in a manner that clearly involved an overcompensation to an initial toxic response. This type of dose–time–response was also reported by others such as Professor Elizabeth **Smith (1935)** of the University of Wisconsin who reported that UV radiation induced a biphasic dose response for mycelium growth in which the stimulatory response occurred only after the UV-induced initial damage with a rebound stimulation reflecting the overcompensation response. Large numbers of similar overcompensation stimulation dose responses have been reported and summarized (**Calabrese and Baldwin, 2003**).

Of significance was that the reporting of a low-dose stimulation after an initial toxicity was viewed by some as a refutation of the hormesis hypothesis. This was particularly the case in the area of radiation biology. For example, while Manfried Fraenkel argued that low doses of ionizing radiation can stimulate biological processes by a direct positive effect (**Josephs, 1931**), Holzknecht and Pordes rejected the possibility of a direct stimulatory response without an initial induced damage (**Josephs, 1931**). The confusion over whether the Arndt–Schulz law was the result of a direct response or a phenomenon following a response to damage became an important conceptual battle that was still evident several decades later. This dispute was important since it attracted many leading researchers in the field of radiation and its medical applications such as Holzknecht, a former colleague of Röentgen and the person recognized as having created the first method of quantifying X-ray exposure. He was also the first European professor of medical roentgenology (**Josephs, 1931**). The lack of both resolution and understanding of the concept of hormesis also eroded its acceptance as the rapidly maturing field of radiation biology/medicine entered the 1940s. This issue was highlighted when the prestigious Harvard professor and first director of the Division of Biology and Medicine at the US Atomic Energy Commission, **Warren (1945)**, continued to promote the concept of Holzknecht and Pordes with comments that the "assumption that small doses of X-ray or radiation are stimulatory (the Arndt–Schulz "law") is invalid. The slight evidences of proliferative activities offered as evidence by the proponents of this hypothesis are in fact only reparative responses to the injury that has been done." Warren would continue to provide considerable leadership to the field, serving on the first US NAS BEAR Committee in 1955–56, being the chair of the pathology panel and a member of the genetics panel that recommended a switch from a threshold to a linear dose–response model for risk assessment purposes.

The rejection of the Arndt–Schulz law by key leaders in the radiation community such as Warren over the fact that radiation often induced stimulation via an overcompensation response following damage was a significant judgment leading to the continued marginalization of the hormesis concept. These leaders failed to grasp that radiation and chemicals had the capacity to induce stimulatory responses at low doses via either direct or overcompensation processes. They also failed to recognize that the quantitative features of these dose responses were similar regardless of the means of stimulation induction. In fact, it is particularly ironic that now, more than seven decades following such marginalizing judgments, the definition of hormesis incorporates the overcompensation response following a disruption in homeostasis concept along with a direct stimulation component (**Calabrese, 2008a**). This overcompensation stimulation concept of hormesis is the same definition that was rejected by leaders such as Holzknecht and Warren. It therefore seems that these early leaders within the radiation community had derived a clear scientific understanding of the overcompensation concept but marginalized it to the point that it was not considered a significant biological phenomenon. In fact, the overcompensation stimulation concept of hormesis, which was rejected due to its lack of apparent biological relevance, evolved into a modern biological/toxicological hormetic mechanism theory by **Stebbing (1982,2009)** by the late 1970s involving various feedback compensatory mechanisms.

Even when the stimulatory response was the apparent result of a direct stimulatory response, it was often not considered of particular importance. For example, the widely cited publication of **Marshall and Hrenoff (1937)** emphasized that the stimulatory response to disinfectants "is frequently of no practical value." The inclusion of the stimulatory dose range for agents such as disinfectants was for illustration of the completeness of the entire dose–response spectrum rather than for its biological significance.

Even the well-known bacteriologist **Rahn (1932)** modeled the hormetic biphasic dose response. He noted that this model was in fact widespread and generalizable. Importantly, he offered a mechanism, involving an enzymatic explanation for the low-dose stimulatory response. Using an example of the effect of arsenic on zymase activity, he suggested that the toxic agent most likely acts as a catalyst, enhancing enzyme activity along with enzyme degradation. He proposed that there was a shifting of the optimum enzyme activity with time from higher to lower concentrations of the toxic agent. While Rahn offered an early biostatistical model-based framework to assess biphasic dose responses, this work, like that of many other investigators, failed to emerge and thrive during the first half of the 20th century, in contrast to its dose–response rivals.

While some of the blame for the failure of the hormesis concept to thrive can be placed on the deliberately deceitful actions of prominent scientists such as Clark (**Calabrese, 2011**), a substantial contributory factor to the early demise of the biphasic dose response was due to the lack of leadership and organizational activity of prominent researchers in this area. Further, a detailed assessment of essentially all the leading early hormetic biphasic dose–response researchers has revealed that most redirected their scientific careers to governmental or academic administration or other divergent but compelling research activities (**Calabrese, 2009**). In many ways, the hormetic dose response failed to thrive during this period due to a combination of factors, all of which converged, leading to its continuing marginalization and the exclusion of these findings from the mainstream of science and regulatory application.

The research on hormetic-like biphasic dose–response relationships in the first half of the 20th century was therefore reasonably substantial, competently conducted, and fairly general, affecting a wide range of biological models, endpoints, and agents. It also became clear that the biphasic dose response could occur via a direct stimulation or via an overcompensation to an initial disruption of homeostasis. Despite these general findings, the concept kept being tied to homeopathy due in large part to both the work of Schulz and the misrepresentations of Clark.

The Resurgence of Hormesis

While these historical issues impeded the development of the hormesis concept, this concept would become transformed during the 1970s, principally lead by the work of three researchers: **Luckey (1980,1991)**, a biochemist, visionary, and leader in the area of radiation hormesis; **Stebbing (1982)**, a marine toxicologist, assessing the effects of toxic metals on marine life; and the research of the pharmacologist **Szabadi (1977)**, who developed a receptor-based mechanistic explanation of hormetic dose responses. Their efforts were significant for multiple reasons. In the case of Luckey, he devoted nearly six decades of research to hormesis, with the notable contribution of the first book published on this topic in 1980. Luckey was particularly important since he was the first to assemble and integrate a vast array of scientific literature on radiation hormesis. Luckey would also inspire the first conference on the topic of hormesis that was held in August 1985, in Oakland, California. While Luckey had planned to do a similar book on chemical hormesis, he finally decided to update his initial book on radiation hormesis about a decade later (**Luckey, 1991**).

These three individuals provided the intellectual bridge that enabled the concept of hormesis to break from historical impediments, such as its close identification with the medical practice of homeopathy, and to become mainstreamed into the core of biological and biomedical research. Along with the reestablishing of the biphasic dose response as an experimental and testable hypothesis, this concept would also be renamed, receiving the moniker hormesis from the Greek word meaning to excite by Chester Southam and John Ehrlich, fungal experts at the University of Idaho. Their 1943 publication reported that extracts from the red cedar tree biphasically affected the growth of multiple fungal strains (**Southam and Ehrlich, 1943**). These researchers were aware of the copious earlier microbiological findings in this area but offered the name change anyway. It is interesting to note that they first proposed the name of hormesis in an unpublished undergraduate thesis by Southam in 1941 in which the term toxicotrophism was proposed to describe this biphasic dose–response phenomenon. **Table 1** provides the quote from **Southam's (1941)** undergraduate thesis. As can be seen, the term hormesis was neatly printed over the crossed out and now forgotten toxicotrophism term. The hormesis term would eventually be adopted by leading indices such as PubMed and Web of Science and used by many researchers, replacing its historical precedent, the Arndt–Schulz law.

Despite the efforts of Luckey, Stebbing, and Szabadi, the acceptance of the hormetic dose response by the scientific community was slow to materialize. This is evident in the very modest level of citations of leading indices such as the Web of Science in which the term hormesis or hormetic occurred only about 10–12 times per year throughout the entire decade of the 1980s. However, despite this slow research community response to these three transitional figures, the first radiation hormesis conference would spark an interest in the continuation of research on the concept of hormesis. This interest became centered in the activities of a research group at the University of Massachusetts at Amherst via the biological effects of low level exposures initiative that would provide the missing leadership, organization, and scientific focus on the topic. As a result, regular conferences were held on the topic, a peer-reviewed journal (i.e., Dose Response) was created, and renewed visibility was achieved that lead to a broad swell of funded research on the topic worldwide. The net result is that citations in the Web of Science progressively increased since the late 1990s now passing 19,000 citations for 2022 alone.

During this period, a number of important developments occurred, which reversed the long-term marginalization of hormesis. These centered on the creation of multiple hormesis databases using rigorous a priori entry and evaluative criteria. These databases would permit the capacity to estimate the frequency of hormetic dose responses in the toxicological and pharmacological literature and provide the capacity to assess the generality of the hormetic dose response, across the plant and animal kingdoms, numerous cell types, inducing agents, and endpoints measured. These efforts also revealed its quantitative features, its occurrence in the toxicological and pharmacological literature and at multiple levels of biological organization, and its mechanistic bases (**Calabrese, 2010; Calabrese and Blain, 2005, 2009, 2011**). **Fig. 1** provides a series of hormetic dose responses, illustrating the generality of the response across biological model and endpoint and the general consistency of its quantitative features.

What was learned in these initial attempts to validate the hormetic dose response was striking and unexpected. Such efforts surprisingly revealed that the threshold dose–response model that was adopted in the late 1920s and 1930s by many regulatory agencies had never been validated or vetted for its capacity to predict responses below the toxicological or pharmacological threshold. The threshold dose response was merely assumed to be correct, and numerous regulations were based on this assumption. This created the opportunity to compare the capacity of the threshold, linear, and hormetic models to accurately predict responses in the low-dose zone. Thus, an effort was then made to vet these three dose–response models and assess their performance in fair head-to-head comparisons. Multiple large databases were either created or obtained that provided opportunities for the intermodel prediction assessment. Such testing revealed that the only dose–response model to make consistently accurate predictions across all evaluations in the low-dose zone was the hormetic dose response. Both threshold and linear dose responses uniformly performed very poorly regardless of the biological models used, chemicals tested, and endpoints measured (**Calabrese et al., 2006, 2008, Calabrese et al., 2010**). These findings are significant since they challenge the validity of the current regulatory approaches, which are entirely dependent upon dose responses that had never been adequately validated. These findings are as striking as they are important as the issue of historical model validation had not been previously raised by the regulatory, medical, or toxicological communities throughout the 20th century for the threshold dose–response model. Thus, despite all the resources devoted to regulatory toxicology by multiple countries during the 20th century, none was directed toward the most critical question of whether the dose–response model that was to be used for risk assessment purposes could actually make accurate predictions in the critical low-dose–response zone.

The issue of model validation was raised in the mid-1970s for carcinogen risk assessment. In this case, the US FDA undertook a massive study of a single well-characterized chemical carcinogen, 2AAF. In this study, over 24,000 mice were used, resulting in the activity being widely called the megamouse study (**Bruce et al., 1981**). The goal of the study was to understand the nature of the dose response in the low-dose zone. Of particular relevance to the subject of this article is that the results indicated that the 2AAF induced a hormetic dose response for renal cancer (**Bruce et al., 1981**). These data were subjected to a detailed analysis by a 14-member expert panel of the *Society of Toxicology*. This group confirmed that the dose response for the key renal tumor endpoint was indeed biphasic, with a significantly reduced tumor incidence at low doses, thereby supporting the hormesis hypothesis in the largest rodent cancer bioassay ever undertaken (**Fig. 2**).

These collective studies revealed that the dose–response models used by the regulatory agencies for public health protection have significant limitations and were not properly vetted over the nearly 50 years since the creation of EPA and OSHA. It is also ironic that **OSHA (1980)** would organize and conduct massive carcinogen regulatory hearings and yet fail to adequately address the issue of dose–response model validation.

Besides performing poorly in the direct head-to-head comparisons with the hormesis dose–response model, the multiple additional hormesis databases provide extremely large numbers of hormetic dose responses that reveal further and substantial instances in which the standard default models used by the regulatory agencies repeatedly fail to predict dose–response relationships in the low-dose zone. The question must be raised as to how often can these default regulatory models be inconsistent with the peer-reviewed published literature before regulatory agencies correct such limitations? These findings and the failure to adequately address such issues undercut regulatory strategies for standard setting for multimedia agents, affecting drinking water, air, soil, and food.

The vast majority of hormetic dose responses display maximum amplitude of less than twice the control group value, with the strong majority being within the range of 30–60% greater than the control group (**Calabrese et al., 2019**) (**Fig. 3**). The dose range for the hormetic dose response is more variable. The strong majority of hormetic dose responses display a stimulatory range of 5–20-fold immediately below the estimated threshold. However, the stimulatory range could be substantial with about 5–10% of dose responses in the hormetic database having a stimulatory range exceeding 1000-fold (**Fig. 4**; **Calabrese and Blain, 2011**). The reason for the variable hormetic stimulatory zone range is not known. However, it may be related to the degree of genetic heterogeneity of the study population and the endpoints being measured and perhaps the possibility of developmental windows of responsiveness/susceptibility that may be quite narrow, thereby creating conditions for wide variation with respect to the dose range of the hormetic dose response.

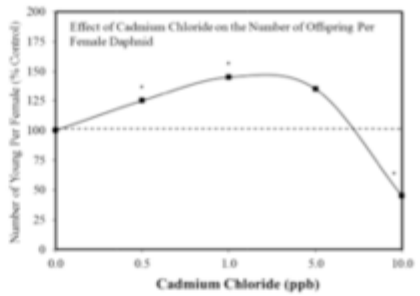
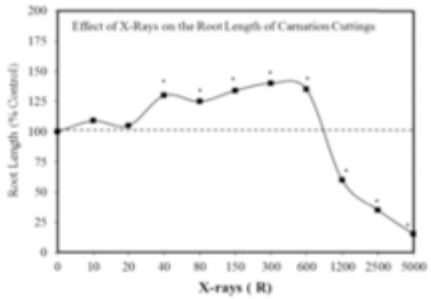
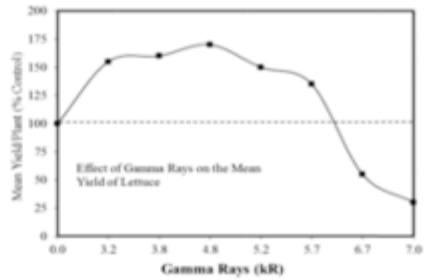
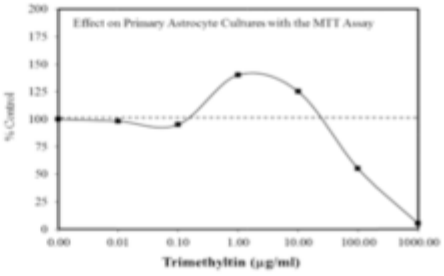
Reference	Hormetic Dose Responses
Bodar et al., 1988	
Bors and Zimmer, 1970	
Bottino and Sparrow, 1973	
Cookson et al., 1995	

Fig. 1a Examples of hormetic dose responses showing biological generality and dose–response features. Adapted from Calabrese, E.J., 2005. Paradigm lost, paradigm found: The re-emergence of hormesis as a fundamental model in the toxicological sciences. Environmental Pollution 138, 379–412.

The most striking characteristic of hormetic dose responses is that the magnitude of the low-dose stimulation is modest and highly constrained and that this is a feature that is independent of model, endpoint, inducing agent, age, gender, and mechanism. This finding suggests that the value that could be contributed by dose–response modeling for hormesis responses in the low-dose zone would likely be limited. Various groups have developed biostatistical models of hormetic dose responses (**Beckon et al., 2008; Belz and Piepho, 2012; Murado et al., 2011; Zhu et al., 2013**), but to date, these modeling efforts have had a limited impact on enhancing biological understandings or on various possible applications. Of significance is that the hormesis dose response offers a different dose–response framework for evaluation as compared with linear dose–response modeling in which estimates of risks at very low doses could be profoundly different, at times up to a 106-fold, depending on the model. This striking problem with model estimate differences for cancer risk

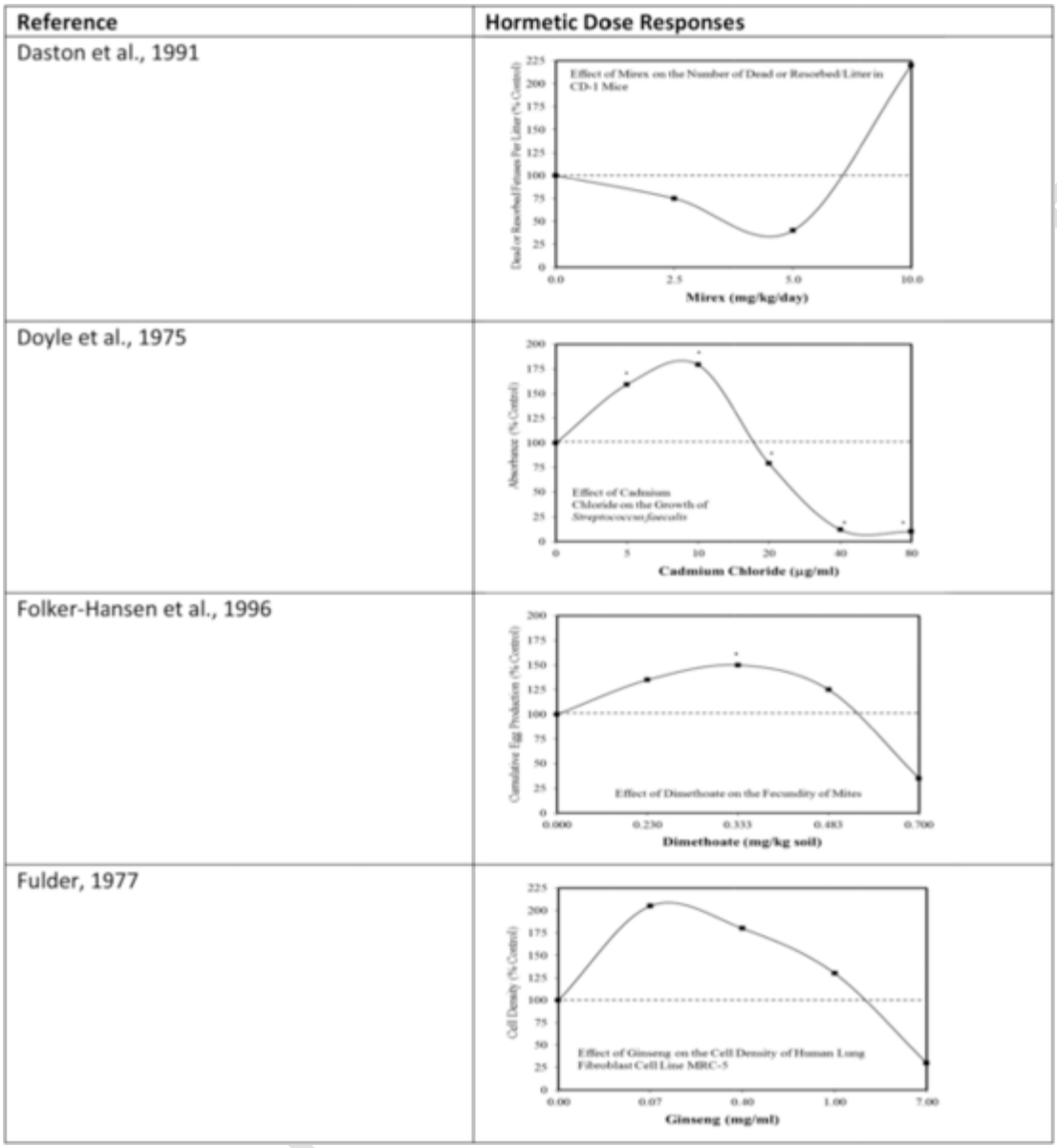


Fig. 1b .

assessment for LNT modeling for very low risks is therefore not an issue in the case of hormesis regardless of the endpoint. Further, cancer risks in the hormetic dose–response framework would be about two orders of magnitude lower than that with LNT modeling. Since hormetic benefits are likely to emerge below about 1% cancer risk, there would be relatively small differences in risk estimates among most viable cancer risk assessment models down to this risk level. Thus, the adoption of the hormetic model has the potential to transform cancer risk assessment, possibly affecting animal model selection, study design, sample size, and use of biostatistical modeling and underlying dose–response assumptions.

Sielken and Stevenson (1998) assessed the implications for quantitative risk assessment assuming the possibility of a hormetic dose response. For example, they noted that dose–response models that are fit to both bioassay and epidemiological data should no longer be constrained to have the probability of the adverse health effect always linearly increasing at low doses. This nonnegativity restriction of the fitted multistage model should be dropped in order to permit the fitted model to better model the shape of the actual dose–response data. Such changes would provide the multistage model with far greater flexibility to reveal possible hormetic effects. Thus, the functional forms and parameterizations of dose–response models need to be expanded to permit hormetic effects to be incorporated into the fitted dose–response models. These authors also addressed in some detail how a consideration of hormesis would affect the issue of the number of doses, dose selection and spacing, and the case of interim sacrificing in order to assess dose–time dependence and hormetic responses.

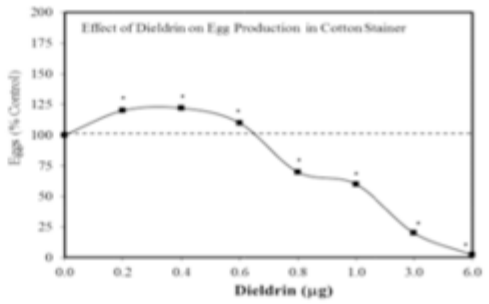
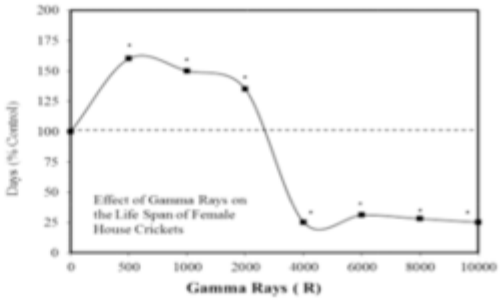
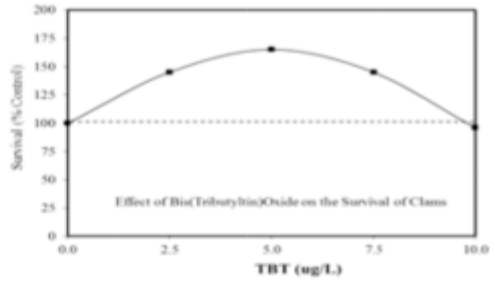
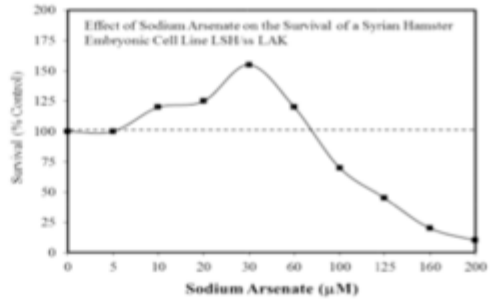
Reference	Hormetic Dose Responses
Hodjat, 1971	
Hunter and Krithayakiern, 1971	
Laughlin et al., 1989	
Lee et al., 1985	

Fig. 1c .

In fact, it was the use of dose–time dependency in the megamouse study that revealed the striking hormetic dose response (**Bruce et al., 1981**).

The hormesis databases have indicated that such biphasic dose responses are reported in essentially all experimental models, including a broad range of plant species, a similarly broad spectrum of microbiological models, such as various species of bacteria, fungi, protozoa, yeasts and other microbes, numerous invertebrates and vertebrates, and humans. It appears that most, if not all, experimental models would be expected to display hormetic dose responses within some experimental contexts. That is, hormetic effects are an evolutionary expectation and highly selected for. The hormetic dose response also occurs in vitro and in vivo and at various levels of biological organization, being observed within cells, organs, and whole organisms. The various hormetic databases also reveal that the hormetic response is also independent of the endpoint measured. Of particular interest and significance is that hormetic dose response is also independent of

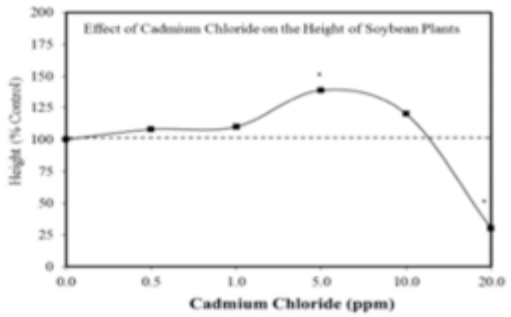
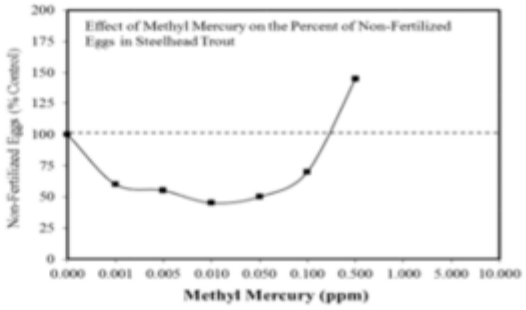
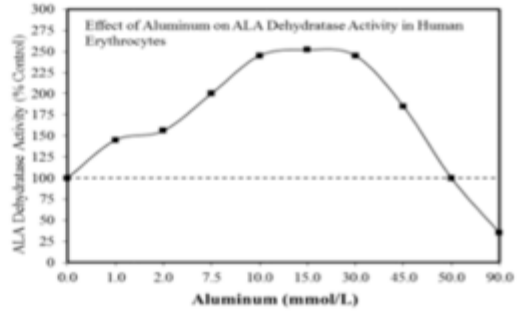
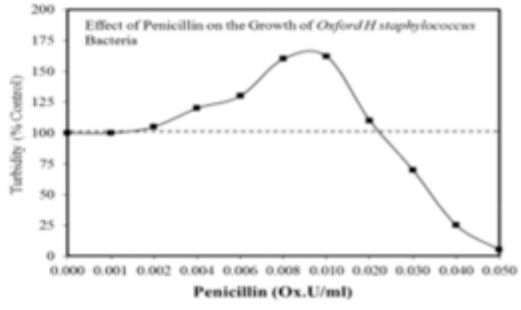
Reference	Hormetic Dose Responses
Levings, 1977	
McIntyre, 1973	
Meredith et al., 1977	
Miller et al., 1945	

Fig. 1d .

mechanism. This is of particular significance as the EPA typically demands that mode of action/mechanism be understood before impacting regulatory actions. This is the case since knowledge of mechanism can be useful in predicting the shape of the dose response for toxic agents at relatively high doses (**Slikker et al., 2004a,b**). However, this does not appear to be the case for hormetic dose responses, in which the magnitude of the stimulatory response is not significantly affected by mechanisms that mediate the low-dose stimulatory response. **Calabrese (2013a)** published mechanisms of 400 hormetic dose–response relationships in which receptors and/or cell signaling pathways were unequivocally shown to mediate the response. This large body of information revealed that even though many specific re-

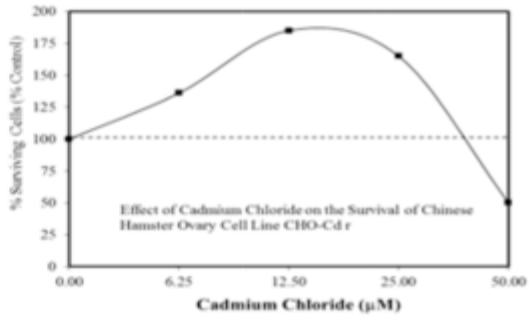
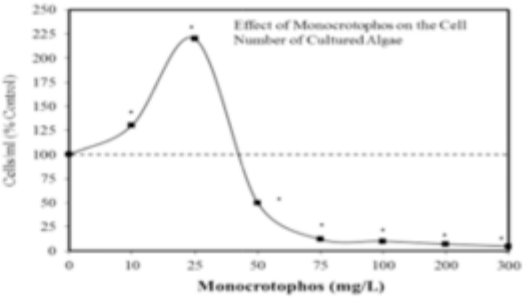
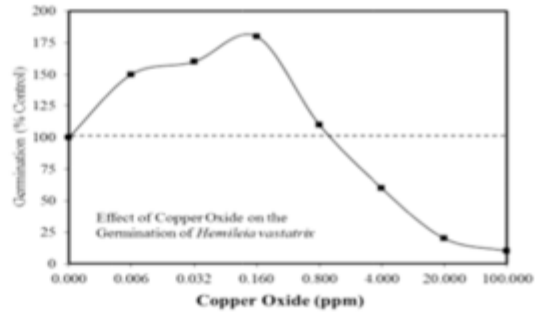
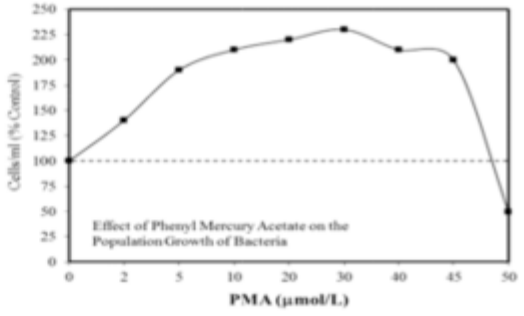
Reference	Hormetic Dose Responses
Monia et al., 1987	
Nayak et al., 1996	
Nutman and Roberts, 1962	
Pahan et al., 1993	

Fig. 1e

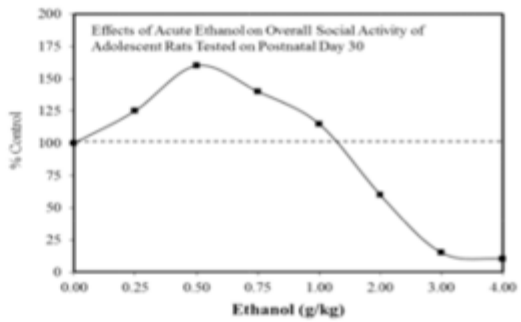
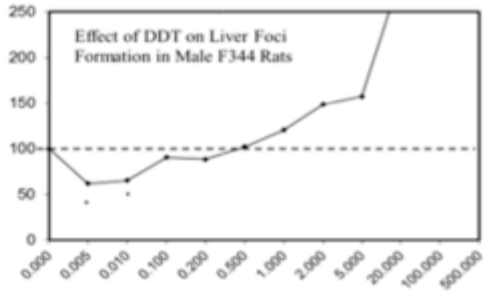
Reference	Hormetic Dose Responses
Varlinskaya et al., 2001	
Sukata et al., 2002	

Fig. 1f .

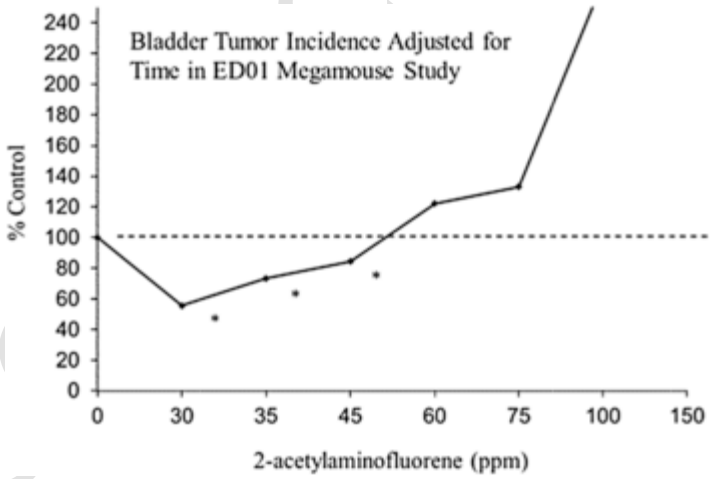


Fig. 2 Bladder tumor incidence adjusted for time in ED01 megamouse study. Adapted from Bruce, R.D., Carlton, W.W., Ferber, K.H., *et al*., 1981). (Members of the Society of Toxicology ED01 Task Force). Reexamination of the ED01 study: why the society of toxicology became involved. Fundamental and Applied Toxicology 1, 26–128.

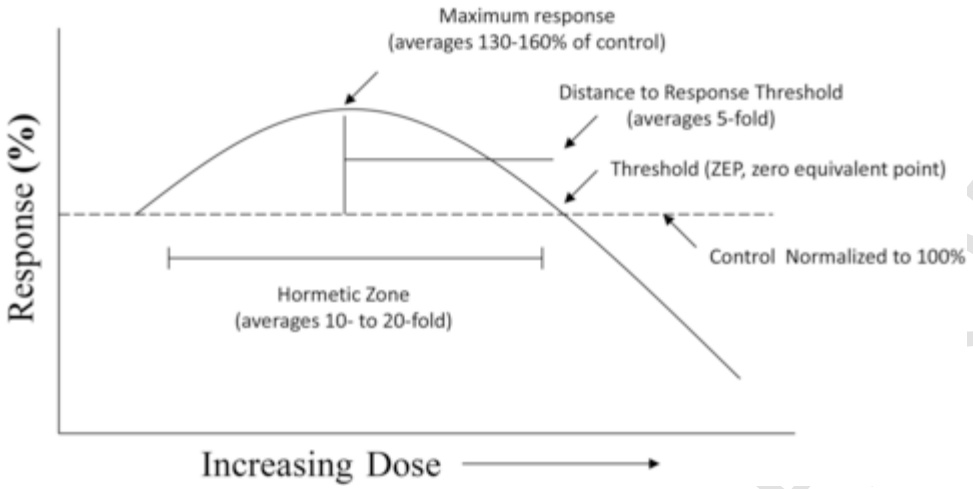


Fig. 3 Dose–response curve depicting the quantitative features of hormesis. Adapted from Calabrese, E.J., Baldwin, L.A., 1997. A quantitatively-based methodology for the evaluation of chemical hormesis. Human and Ecological Risk Assessment 3, 545–554.

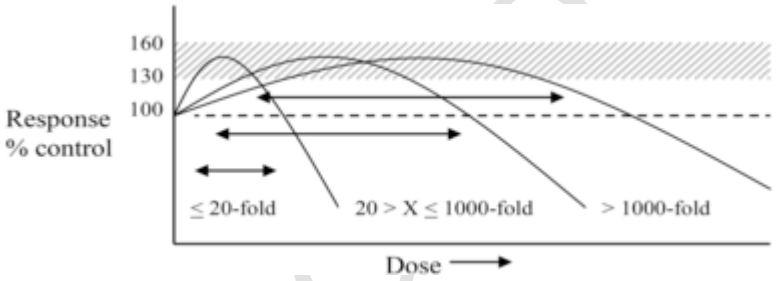


Fig. 4 Dose–response curves indicating that even though the maximum stimulatory hormetic response is typically only 30–60% greater than the control response, the range of the stimulatory response may exceed several orders of magnitude of dose even though it is generally observed to be less than 20-fold. Adapted from Calabrese, E.J., Baldwin, L.A., 2002b. Applications of hormesis in toxicology, risk assessment and chemotherapeutics. Trends in Pharmacological Sciences 23, 331–337.

ceptors and pathways were involved in mediating this highly diverse range of hormetic dose responses, the quantitative features of the responses were similar regardless of the receptor, pathway, or various types of receptor and pathway interactions.

Beneficial Versus Harmful

While considerable attention was directed to historical debates over whether the low-dose stimulation was the result of a direct or overcompensation response, with the answer being that both satisfy the definition, a debate arose in the 1980s that hormetic effects were beneficial effects only. Detailed evaluations of this issue revealed that hormesis should be viewed as a dose–response phenomenon, independent of whether the response could be beneficial, harmful, or perhaps of no yet known clinical or biological significance. Considerable evidence exists in which hormetic effects offer unequivocal benefit to the individual as in cases of life extension, decreases in damage due to heart attacks or stroke, and increasing memory performance, among many other seemingly clear examples of benefit. Numerous examples of hormesis also exist in which one might interpret the effects as undesirable as in cases of low-dose enhancement of tumor cell proliferation, enlargement of the prostate gland, enhancement of some autoimmune functions, and others (**Calabrese, 2008a,b; Calabrese et al., 2013**). There are also substantial examples of hormetic dose responses in which the clinical significance remains to be determined.

Whether hormesis could induce protective effects from agents recognized as harmful such as cadmium, lead, mercury, dioxin, cigarette smoke, ionizing radiation, or other such agents has been widely discussed. It has long been known that agents such as cadmium, lead, and ionizing radiation can enhance the growth of various plant species at low concentrations (**Calabrese and Baldwin, 2000a,b**). At very low doses, Pb is a mitogen in the rat liver (**Calabrese and Baldwin, 1992**), stimulates immune response in mice (**Koller et al., 1979**), and stimulates erythropoiesis (**Iavicoli et al., 2003**). It is worth noting that methyl mercury, a powerful neurotoxin and even

more so than lead, enhanced the viability of a neuronal cell line, clearly displaying a hormetic dose response (**Toimela and Tähti, 2004**; **Fig. 5**). Further, considerable research has also demonstrated that ionizing radiation at low doses can induce a plethora of beneficial effects in multiple and far-ranging biological models from plants to people (**Calabrese and Baldwin, 2000a,b,c,d,e; Calabrese and Calabrese, 2013a,b**) and for a wide range of endpoints. These findings not only challenge current investigations about the nature of the dose response in the low-dose zone but also indicate the need to assess the entire dose–response continuum in order to conduct a more definitive hazard/risk assessment and to derive an improved evolutionary understanding of the role of background levels of heavy metals, secondary metabolites, ionizing radiation, and other agents in organismal biology. Thus, low-dose hormetic effects could be beneficial or harmful and need to carefully be assessed.

Synergism and Hormesis

The occurrence of chemical interactions is an important issue in toxicology and pharmacology (**Calabrese, 1990**). These are manifested within the context of additive, synergistic, and antagonistic responses. It has been commonly asked whether hormetic dose responses display such interactions. There have been a limited number of studies that have addressed the issue of interactions between multiple hormetic agents within the low-dose stimulatory zone. Such research has permitted some insight on this topic. The most significant of these studies have been conducted in the area of drug-induced enhanced learning and memory (**Calabrese, 2008a; Flood et al., 1983, 1985**). In general, such studies have tested numerous dose combinations in which there have been various degrees of low-dose stimulatory responses. These findings revealed that regardless of the degree of stimulation of an individual agent, the maximum response was limited to the 30–60% greater than the unexposed control group. Numerous dose combinational experiments following detailed grid-like dose matrix testing schemes revealed the occurrence of additive and/or synergistic responses between the various agents. However, the maximum responses in any of these combinational drug dose studies designed specifically to affect an optimal response only approached the 30–60% stimulatory zone, as a ceiling response seemed to be reached. Any enhancing interaction observed at the lower doses markedly diminished as the responses approached the 60% greater than control value. Thus, synergistic responses occurred but only at the very low doses. However, even these interaction-mediated responses were constrained by the limits of plasticity (30–60%) as were the responses of individual agents.

These findings are different than that observed with toxic agent-induced synergistic responses where very substantial increases in toxicity may occur. These truncated and limited hormetic interactional phenomena have significant biological, biomedical, and clinical implications, indicating that the maximal hormetic enhancements appear to be restricted to a ceiling response of about 30–60%. This would also apply to situations in which the low-dose stimulation is undesirable as in the case of drugs enhancing tumor cell proliferation. Thus, the biology of low-dose responses within the hormetic zone displays a fundamentally different behavior than seen within a more traditional high-dose toxicological framework. Since this perspective is of considerable risk assessment significance, it needs to be incorporated into the formal framework for agent assessment.

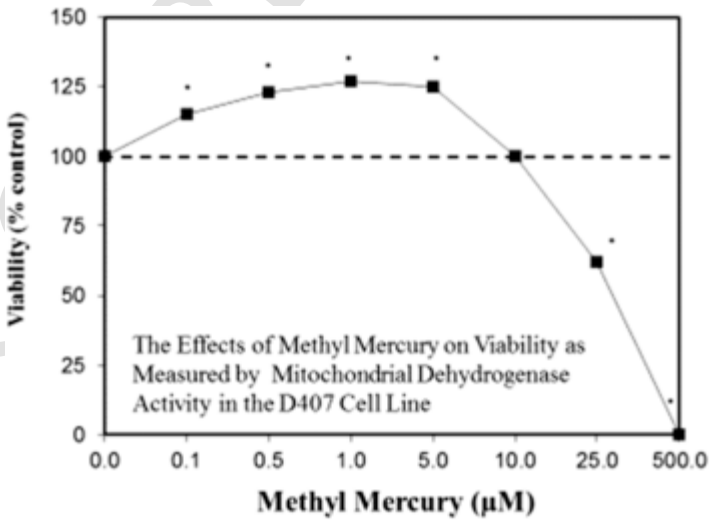


Fig. 5 The effects of methyl mercury on viability as measured by mitochondrial dehydrogenase activity in the D407 cell line. Adapted from Toimela, T., Tähti, H., 2004. Mitochondrial viability and apoptosis induced by aluminum, mercuric mercury, and methylmercury in cell lines of neural origin. Archives of Toxicology 78, 565–574.

Hormesis and the Limits of Plasticity

Regardless of the biological model, the endpoint-inducing agent, and mechanism, the maximum stimulation for hormetic dose responses is usually within the 30–60% zone greater than the control group. These responses, therefore, are highly conserved across all models thus far studied, from plants to microorganisms and for invertebrate and vertebrate models. This raises the very interesting evolutionary question as to why this is the case. This is an area that has yet to be addressed adequately by the evolutionary biology community. However, the limited maximum stimulation of hormetic dose responses is the most striking feature of this dose–response phenomenon. The extreme commonality of this limited stimulatory response suggests that the maximal stimulatory response is governed by the constraints of biological plasticity. It also suggests that the limits of plasticity are similar across all the phyla. This is a significant observation and indicates that hormesis provides the first estimation of the quantitative expression of plasticity. It also provides a new and expanded concept of the plasticity concept (**Calabrese and Mattson, 2011**).

Preconditioning

Preconditioning/adaptive responses are commonly reported in toxicology and pharmacology. This is a phenomenon in which a prior low-dose exposure to a stressor agent may protect against a subsequent more massive exposure to the same, similar, or different agents. This phenomenon has a long history, being first reported by **Ancel and Lallemand (1928)** in extensive research involving some 48 independent experiments showing that low doses of X-rays protected plants from the toxic effects of subsequent high doses of X-rays. This observation would be extended by numerous investigators in the domain of ionizing radiation using plant and animal models and multiple endpoints (**Calabrese, 2016a,b**). In a remarkable series of papers in the 1950s, **Pape (1950,1951)** and **Pape and Jellinke (1950)** provided what may be the first detailed graphing of the preconditioning findings that show clear evidence of a hormetic dose response. Preconditioning was generalized to the domain of chemical toxicology in the 1960s with research on heavy metals (**Ito and Sawauchi, 1966**) and the effects of chlorinated solvents such as CCl_4 (**Dambrauskas and Cornish, 1970; Glende, 1972; Ugazio et al., 1972, 1973**) and by the 1970s to the area of chemically induced mutations (**Samson and Cairns, 1977**).

As research in this area expanded and more experimental parameters were assessed, it was observed that the preconditioning phenomenon consistently displayed a hormetic dose–response pattern, with similar quantitative features as seen with the many thousands of examples of hormesis that did not concern preconditioning (**Calabrese, 2016a,b**). It was also shown that the optimal protection after a challenging dose was induced by the same conditioning dose that induced the largest adaptive mechanism response during the conditioning period, prior to administration of the challenging dose. Blocking of the inducing mechanism during the conditioning time period also resulted in blocking the protective effect after the challenging dose. Such temporal, mechanistic, and optimal dosing responses were therefore integrated within a framework that established that preconditioning is a specific type of hormetic dose response. This is an important development since preconditioning has a vast array of clinical and public health applications that can be directly linked with the predictable nature of its quantitative features. This constitutes a considerable advance for estimating beneficial and perhaps harmful effects due to the hormetic dose response.

Hormesis and Chemical Interactions

It is generally assumed that most toxic substances interact in an additive way. This may be because a large proportion of the studies published on the topic involved experimental protocols in which the joint chemical exposures were typically given in a concurrent rather than in a sequential time framework with various windows of exposure. Within this context, it is generally recognized that lead and mercury are renal toxins. Consequently, it would be typically assumed that exposure to these agents might have the potential to adversely affect kidney toxicity in an additive manner. However, a study conducted by **Ewald and Calabrese (2001)** introduced a temporal component in which a low dose of lead was administered to mice a day before a renal toxic dose of inorganic mercury. Rather than an additive toxicity effect, a profound reduction in renal toxicity was observed. This type of study design would be an example of a preconditioning hormetic dose response. The issue therefore of chemical interaction should be carefully framed within a temporal window and dose consideration as numerous experimental conditions could be created in which hormetic dose responses might be expected.

In addition to preconditioning having important potential clinical and public health applications, it also has implications for the risk assessment process. More specifically, in the case of carcinogen risk assessment, it has long been assumed that carcinogens follow a linear dose response. This linear dose–response model assumes that the effects (e.g., mutations) are harmful, cumulative, and irreversible. This suggests a concentration × time = constant relationship. However, the concept of preconditioning illustrates that for large numbers of agents, endpoints, and studies, the LNT mantra cannot be correct. That is, preconditioning research provides a vast domain of biological responses for which the cumulative and the irreversible components of the LNT model are not valid. Since exposures during life are sequential, often intermittent, and highly variable, it suggests that preconditioning processes may impact the nature of the response from toxic and carcinogenic substances.

Prior exposures to toxic, mutagenic, and carcinogenic agents within the correct temporal window have the capacity to diminish the effects of a subsequent more toxic and/or carcinogenic effect. In fact, the hormetic dose–response model would predict that such preconditioning effects would affect a 30–60% decrease in the harmful effects due to the subsequent exposure and/or extend latency periods (**Carlisle et al., 2010**). This concept was never considered in the derivation of exposure standards for toxic and carcinogen agents. In fact, they were assumed not to exist.

The regulatory community assumes that exposure to carcinogens follows an additive to background process. It needs this assumption to support the LNT mantra. In fact, this is precisely what the US National Academy of Sciences Safe Drinking Water Committee recommended in their landmark book entitled *Drinking Water and Health* in 1977. Despite a voluminous number of scientific publications that demonstrate exceptions to this generally accepted assumption, no attempt was ever made to integrate such limitations into the risk assessment process. Even in the four decades since the NAS recommendation and the explosion of research on preconditioning, no attempt has been made to update and revise this key assumption used to support the LNT model. This represents another aspect of the risk assessment process that is flawed, in this case because it failed to integrate the preconditioning concept.

Risk Assessment, Adaptive Response, and Hormesis

The goal of the risk assessment process according to the EPA is to prevent pollutant-induced harm. However, this goal is made more interesting by the second half of the EPA definition of a risk assessment. That is, according to EPA, the risk assessment process should not consider whether or not there are possible health benefits due to exposure. In their own words, the EPA indicates that "as the purpose of a risk assessment is to identify risk (harm, adverse effects, etc.) effects that appear to be adaptive, non-adverse, or beneficial may not be mentioned." This definition of the EPA represents a framework whereby the hormesis effect would be not only marginalized but also actually discounted. The irony is that EPA could recognize the occurrence of adverse effects related to a hormetic biphasic dose response as in the cases of an increase in prostate size or the speeding up of a developmental process such as puberty. However, it ignores data in which a hormetic response was shown to enhance health by reducing the occurrence of cancer in the population. This dichotomy in perspective and policy reveals that the US EPA concept of risk assessment suffers from a reductionist approach that fails to appreciate that risk and health have a complex interrelationship. The process of risk assessment must recognize that there may be risk from harmful exposures and health risks when benefits are denied. These two perspectives need to be recognized and integrated within a holistic public health-oriented risk assessment process. This integrated view was supported by a strong majority of the US Society of Toxicology in a representative survey of members (**Jones, 2010**).

This discussion illustrates that the EPA regulatory actions do not adequately address the health and wellness needs of society with its too limited view of what a risk assessment is. This has created the need for a type of midcourse alteration that repairs the institutional blind spot that currently exits. Unless such improvements are made, the EPA and many other EPA-like regulatory agencies in large numbers of countries will continue to provide inadequate population-based public health assessments and waste precious public resources. A suggested revised definition of a risk assessment would be a process that estimates the net population disease incidence for each level of incremental exposure. This would ensure that the complex aspects of risks and benefits are properly integrated and the health of the population optimized (**Calabrese, 2011**).

Hormesis Questions and Answers

How Could Hormesis Affect Study Designs?

Hormesis is a dose–response relationship that addresses the entire dose–response continuum. It is not a high dose–few doses approach to toxicology. The hormesis perspective is open to the possibility that biological systems might respond in a fundamentally different way at low doses as compared with high doses. In order to test for this, it is necessary to include a sufficient number of doses with proper dose spacing and statistical power. The dose-spacing issue is important because the width of the hormetic dose response can be highly variable, ranging from < 5- to > 1000-fold. Thus, the process of properly exploring the possibility of having a hormetic dose response may require multiple preliminary experiments to better define the dose–response range and especially the identification of the toxicological and/or pharmacological thresholds. Once the threshold is reasonably well characterized, it would be possible to better explore whether hormetic effects might occur in the low-dose zone. The experimental plan therefore is iterative, with past experiments informing the investigator what approaches and experimental designs to use in the subsequent studies. This is very different from what might be called "cookie-cutter" toxicology in which the experimental approaches are very prescribed, as may often be the case in a regulatory-driven hazard assessment framework.

How Could Hormesis Affect the Hazard Assessment Process?

While hormesis could affect the study design of experiments, it may also affect the choice of animal model. For example, it is common that models selected for use have a very low background disease or tumor incidence. However, in such circumstances, it would not be possible to assess for the occurrence of hormesis. That is, if the model has a near-zero incidence of tumors, it would not be possible to assess whether low doses of the agent being studied could induce a response below that of the control group. This would mean that the ani-

mal model would only be useful for assessing directly the threshold dose–response model and perhaps the LNT response via extrapolation modeling. Since regulatory agency decisions on animal model selection occurred decades before the resurgence of the hormesis concept, this creates a conflict between following the tradition of continuing to use the same animal model and better addressing the nature of the dose in the low-dose zone with a model that permits this possibility. An alternative perspective would be modeling the hormetic dose response in the absence of appropriate data as is done for the LNT risk estimates using standard dosing approaches (**Calabrese et al., 2015a**).

How Many Times is it Necessary to Replicate a Study in Order to Feel Confident That the Low-Dose Hormetic Response is Reproducible?

This is a difficult question to answer as it depends on the control group variability during the study, the historical control group variability response if one exists, the magnitude of the response, and perhaps other factors. In my first encounter of a hormesis dose response, my faculty advisor required me to replicate the experiment eight times, with progressively larger sample sizes and more doses in the low-dose zone, eventually using about 10 doses plus concurrent controls. The magnitude of the response was modest being only about 50% greater than controls. While this degree of replication seemed excessive at the time, there was nevertheless considerable value in knowing that the results were consistently observed. The extent to which one replicates studies, in the end, will depend upon the experience and judgment of the researcher. In many situations today, it is possible at some point to incorporate mechanistic components to the experimental approach. If specific inhibitors can block the low-dose stimulation, it can add confidence to the findings and be used to support judgments concerning the reproducibility of the findings.

How Often is Hormesis Not Seen?

This question is also problematic for a number of reasons. Hormesis can have a time component, requiring multiple measurements over-time. If the time-based observations are not adequate, the hormetic response may be missed. It may also be missed due to high variability in the response parameter and the modest magnitude of the treatment. If there is high heterogeneity within the study population, it could also affect the capacity to detect a hormetic response. There are therefore multiple situations in which a hormetic response may occur, but limitations in study design may preclude the capacity to observe it. On the other hand, there are numerous examples in the literature in which a hormetic response was not apparent even when the aforementioned limitations did not seem to be present. Furthermore, there have been multiple examples in the pharmacological literature in which the hormetic response was dependent upon chemical structure specificity. However, to get back to the original question posed, in several studies by our group in which rigorous a priori entry and evaluative criteria were used, the hormetic dose–responses frequency was about 40–60% in well-designed studies (**Calabrese et al., 2006, 2008, 2010**). These figures would have been considerably higher had the evaluative criteria been modestly relaxed.

What Happens to Hormesis During Aging?

The issue of aging and hormesis is one of considerable interest, especially with respect to preconditioning hormesis. Since the 1990s, evidence has emerged that preconditioning to protect the heart, brain, and other organs can sharply decline in the elderly of multiple species (**Calabrese et al., 2015b**). This is ironic as this would appear to be the time when such hormetic effects would be most needed within modern human society. This has generated much research to explore ways in which such limitations might be overcome. Some insights have emerged suggesting that certain preconditioning functions may be maintained via various exercise regimes and under specified dietary manipulations such as some intermittent fasting protocols.

The aging preconditioning area may also be affected by comorbidities, such as obesity, atherosclerosis, diabetes, and other conditions often also associated with an aging elderly population (**Calabrese et al., 2015b**). In numerous studies, the presence of one or more of these conditions may profoundly decrease the preconditioning protective response, creating a significant public health concern.

Over the past two decades the experimental literature on the biology of aging has been dominated by research with the nematode *C. elegans* because of its short lifespan, ease of rearing, genetic makeup, and vast number of genetic mutants which permit the molecular assessment of pathways and other relevant mechanisms. It has been shown that *C. elegans* commonly displays hormesis in lifespan studies (**Martel et al., 2020**) for numerous agents, including pharmaceuticals, plant based dietary supplements, environmental contaminants as well as for dietary patterns such as caloric restriction. The quantitative features of the dose response with respect to the amplitude and width of the enhanced lifespan response is similar across these agents/treatment, regardless of whether these agents are a single chemical compound or within complex mixtures. Likewise, these hormetic quantitative features are independent of mechanism. These findings appear to have broad implications since the molecular pathways that mediate the hormetic response for health span and lifespan in *C. elegans* are very similar to those in mammalian models. The findings with *C. elegans* are fully consistent with the responses seen in other species, from yeast to mammals. These findings strongly suggest that the capacity to extend life is biologically attainable in experimental systems and is limited by the constraints of biological plasticity which is described by the hormetic dose response (**Calabrese et al., 2023a**).

Hormesis Restoration in Aging and Elderly Subjects

Research on the restoration of degraded hormetic adaptive responses in multiple biological models shows that hormetic-based adaptive activations markedly decline with age and are associated with the onset of a vast range of age-related diseases, in all systems of the body. Some research has been directed toward assessing whether and to what extent degraded hormetic pathways and functions can be restored (**Calabrese et al., 2023b**). While numerous studies have failed to restore age-related degraded hormetic pathways (**Calabrese, 2016c**) several well conducted studies have shown that substantial restoration is possible via both dietary and exercise interventions and their combinations (**Abete et al., 2005; Calabrese, 2016c**). Studies in humans also suggest that hormetic responses can be activated in people greater than 90 years of age as indicated with research concerning recurrent stroke prevention (**Meng et al., 2012, 2015**). It is therefore likely that age-related hormetic research effects will become a major focus of public health research and health promotion activities in the future.

Hormesis and High-Risk Groups

Calabrese and Baldwin (2001) published a detailed assessment of hormesis in the *Annual Reviews of Public Health*. One of the editorial board members sought to explore whether hormetic effects might be expected to occur in those considered at increased risk, as this would be important to know for regulatory purposes. We therefore examined the hormesis database for studies that would permit the evaluation of this question. This effort resulted in the publication explicitly on hormesis and high-risks groups (**Calabrese and Baldwin, 2002a**). It revealed that hormesis often occurred in high-risk groups with their dose response being shifted to the left on the dose–response relationship. Importantly, the quantitative features of the hormetic dose response were similar to that reported for so-called normal segments of the population (**Fig. 6**). Despite the occurrence of hormesis in many high-risk groups, there are examples in the literature in which the hormetic effect may be absent in different life stages or being affected by familial/genetic factors. There is also considerable evidence that many hormesis-adaptive responses are significantly diminished in the elderly (**Calabrese et al., 2015a,b**).

Transgenerational Hormesis

The modest stimulatory hormetic response typically requires the use of sufficient sample sizes to achieve adequate statistical power and the need for multiple doses in the low dose hormetic zone. It is also important that historical control group biological variability is well documented since it is necessary to reliably differentiate treatment responses in the low dose stimulatory zone from background variation. Given the modest magnitude of the hormetic stimulation, it is also often necessary to replicate experiments to ensure reliability of the findings. There is also the need to identify hormetic mechanisms and to use receptor and pathway inhibitors that can block hormetic responses, confirming the hormetic mechanistic process (**Calabrese, 2013a**). This series of steps is necessary to establish the biological foundations of the hormesis concept and to demonstrate its generality within numerous biological systems.

These experimental requirements in the assessment of hormetic dose responses have provided a sound foundation for its application to all phases of the life cycle, from early development through adult maturation and progressive aging to advanced elderly states (**Calabrese, 2015b,c**). The resources and time demands for the study of "generational" or ontological hormesis becomes even greater when

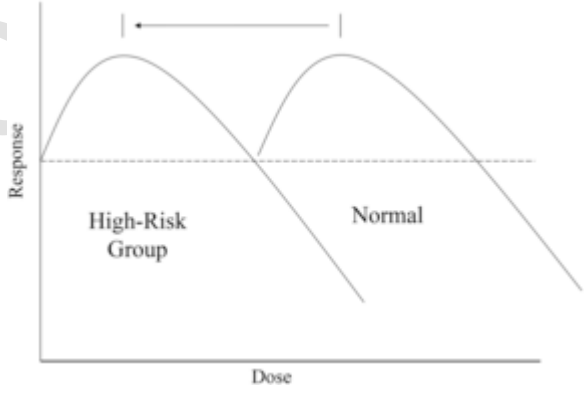


Fig. 6 Dose–response curves of a hormetic response in high-risk groups. This response occurs at lower doses compared with the normal population. Thus, the dose–response curve for the high-risk group is shifted to the left of the dose–response curve for the normal population. Adapted from Calabrese, E.J., Baldwin, L.A., 2002b. Applications of hormesis in toxicology, risk assessment and chemotherapeutics. Trends in Pharmacological Sciences 23, 331–337.

assessing transgenerational hormesis hypotheses. In this context of transgenerational hormesis it is important to utilize well established experimental models, along with robust, hormetic-testing experimental designs and well-defined hormetic-acting agents with known underlying mechanisms. While there is a robust literature on hormetic dose responses, in general, this is not the case for targeted and systematic assessment of hormesis throughout the life cycle. This is because hormesis has been a progressively serious area of research only over the past two decades. In addition, the vast majority of experimental studies with hormetic dose responses have utilized cell culture studies since they are far less expensive and time consuming and can use more concentrations/doses.

Such research greatly favors the use of plant and invertebrate model systems in transgenerational hormetic dose response studies. The published literature on transgenerational hormesis often reflects limited dose response assessments that are targeted for the optimal concentration/dose in the hormetic stimulatory zone across multi-generations. While such approaches are understandable in light of resource limitations the findings also have limited generality of the biological findings. Nonetheless, there are about 60 transgenerational hormesis references cited in the Web of Science database (see **Agathokleous et al., 2021a,b** for reviews). These studies reflect research with both plants and animal models, with the vast focus having been with insect models, especially with the use of multiple aphid species dominating the research. In general, the research has focused on key parameters such as reproduction, fecundity and lifespan. The dosing used has often been limited to 3–4 doses, in general, with the so-called hormetic responses occurring in the relatively low lethal concentration range such as lethal concentration (LC) 5–10 for the induction of transgenerational hormesis. Mechanistic research to date is largely limited to patterns of gene activation without pathway involvement. A particularly well designed transgenerational hormesis study was reported by **Fuciarelli and Rollo (2020)** who employed five treatment groups plus a control group for the first (parental) generation and then three doses from the hormetic zone of the parent generation in the F1 generation using large numbers of crickets. Similar approaches have been successfully shown with various plants species (**Belz, 2020**).

The area of transgenerational hormesis is currently dominated with agricultural applications. However, the fact that transgenerational hormesis is a general phenomenon and should come to profoundly affect the design, conduct and interpretation of human epidemiological studies. In fact, this area has the potential to radically change how epidemiological studies are done and their utility, especially in the medical and regulatory domains (**Belz, 2020; Fuciarelli and Rollo, 2020; Ullah et al., 2020**).

Hormesis and Ultra-Low Doses

The standard dose response in toxicology is actually one of massively high numbers of molecules that are administered to animal models in vivo or to cells in culture. For example, in standard rodent chronic bioassays exposures are typically over tens to hundreds of trillions of molecules per animal/day for the duration of the study. Environmental Protection Agency drinking water standards for chemical carcinogens based on these studies estimate risks of cancer using a linear dose response model, providing risks of one per million people over a lifetime exposure with greater than a trillion carcinogenic molecules/person/day. These observations suggest that organisms and cells are very insensitive to even the toxic agents tested. Despite these important observations, cellular and organismal phylogenetic-based signaling mechanisms have evolved complex dose/concentration-response relationships strategies. The effect(s) of concentration ranges of different agents (ligands and environmental factors) are varied, with most receptor-based and enzymatic patterns operating in the 10^{-6} to 10^{-9} M or 10^{-4} to 10^{-6} M concentration ranges, respectively (i.e., as based on relative dissociation constants). Low doses have typically been viewed as concentrations of a biologically active agent at the site of action that are one or more orders of magnitude lower than the equilibrium dissociation constant (**Gurevich, 2001**). However, emerging evidence of cellular and organismal signaling processes indicates significant biological activity at far lower concentrations, that is, in the 10^{-18} to 10^{-24} M range) (**Calabrese and Giordano, 2021**). In fact, significant biological amplification mechanisms have been reported in which a single molecule can activate transcellular signaling via wave-like bystander communication processes affecting up to tens of thousands of neighboring cells. The present brief summary identifies several areas of ultra-low dose biology (10^{-18} to 10^{-24} M), namely immune responses, such as macrophage phagocytosis (**Roy and Rai, 2004**); hormonal functions [e.g., in Tetrahymena (**Csaba et al., 2007**)]; pressor effects in the renal vasculature (**Dharmani et al., 2005**); pheromonotropic and melanotropic receptor effects in insect species (**Shalev and Altstein, 2015**); sex signaling in algae (**Pall, 1973**); several enzyme activities (**Ershova et al., 2016**); cAMP production (**Civciristov et al., 2018**), and mud crab pheromone pumping factor (**Calabrese et al., 2017**).

While the number of molecules capable of interacting directly with cells in the 10^{-18} to 10^{-24} M range is extremely small, experimental evidence indicates a biological amplification process that is integrated. For example, a ratio of one cAMP molecule/3300 macrophages statistically increased the average phagocytic response by 50%. A similar bystander amplification broadcasting effect was reported by **Offen et al. (2000)** in the neuroprotective actions of two vasoactive intestinal peptide (VIP) derived peptides on PC12 cells, a Parkinson's disease model system. The concentration for both agents at 10^{-18} M (600 molecules/one mL) enhanced the survival of 300,000 PC12 cells by 50–60%, an amplification response of ~ 5000 fold. **Sowa et al. (1997)** demonstrated the capacity of morphine to reduce macrophage phagocytosis at 10^{-20} M by 8.5%, affecting ~3500 cells/molecule (NB: a value similar to that reported for phagocytosis induced by cAMP). These examples of apparent activation of many thousands of cells by a single molecule represent an important conceptual development concerning the ubiquity and potency of hormesis and its applications in environmental science, molecular/cell biology, toxicology and medicine. These examples provide the lowest doses/concentrations tested to date, without evidence of a threshold, thereby suggesting the possibility that similar effects may occur at even greater cell to molecule ratios (e. g., increases in biological activity of in vitro conditions tested involved 300,000 to 1000,000 cells/6–120 molecules).

Several examples of broadcasting effects were assessed in whole organisms (viz., activation of abdominal pumping in the pregnant mud-crab at 10^{-22} M of synthetic peptides, in which significant effects were induced by an estimated 2–3 molecules per crab (**Pettis et al., 1993**)). Similar heightened sensitivity was reported in the green algae, Volvox, which displays both sexual and asexual reproduction. In 1973, Pall reported sexual induction as a function of hormone concentration. Based on studies with *Volvox carteri*, it was determined that only two molecules of hormone are required to make the gonidium form a sexual rather than an asexual embryo. These findings suggest that such ultra-sensitive signaling is a common and broadly generalized evolutionarily based survival strategy. This is strengthened by the work of **Civciristov et al. (2018)** in citing 35 established examples of ultra-sensitive detection/signaling in a variety of biological systems. It is possible that ligand ultra-sensitivity may be important to establishing (cellular and organismal) responsivity to various environmental niches and conditions, whereby ultra-low dose sensitivities may enable hormetic signal amplification in relatively "noisy" environments, thereby creating more resilient phenotype(s) that are more allosterically capable, and adaptable (**Calabrese et al., 2017**). These new findings show that hormetic dose response strategies are also independent of the range of effective dose response relationships. These novel hormetic findings are likely to expand the range of biological and toxicological possible effects in the ultralow dose areas that are physiologically significant.

Nrf2: Hormetic Stress, Redox Signaling, Antioxidant Response, and Inflammation

All cells are continually exposed to reactive oxygen species (ROS), including superoxide, hydrogen peroxide and hydroxyl radicals (see Chapter 1.14 in this volume). For example, ample amounts of ROS are continuously produced by the electron transport system (ETS) of mitochondria during aerobic respiration and by other biochemical reactions and in other organelles. Environmental stressors, such as xenobiotics, UV light and ionizing radiation, also contribute to and enhance ROS formation. Together, the sum of oxidative stresses may upset the delicate redox balance and drive cells toward pathologically pro-oxidant as well as pro-inflammatory states. To maintain redox homeostasis and prevent deleterious effects due to excessive oxidative stress, cells utilize slight increases in ROS as signals to upregulate the production of antioxidant enzymes and molecules that may then scavenge oxidants, restore redox balance, and prevent oxidative damage. Nrf2 is a redox-activated transcription factor (TF) that functions not in isolation but in concert with other TFs, including p53, NF-kB, and Keap1. If Nrf2-mediated antioxidant responses are insufficient to reduce oxidative stress then one TF in particular, NF-kB, is activated and upregulates pro-inflammatory responses, such as TNFα, which may be pathological when chronically sustained or beneficial when acutely directed at infectious microbes or cancer cells. It is the redox signaling and crosstalk between Nrf2 and NF-kB, including the interactions of other TFs, that work in concert and assure that beneficial and not the pathological manifestations of anti- and pro-inflammatory effects occur (**Ahmed et al., 2016; Yeang et al., 2012**). In mammalian cells, the Keap1 protein is a key redox sensor of intracellular ROS (**Motohashi and Yamamoto, 2004**). At normal redox homeostasis, Keap1 sustains the ubiquitination and degradation of Nrf2 to which it is bound, keeping Nrf2 at low levels. However, with increases in exposure to subtoxic (i.e., hormetic) levels of oxidative stressors, several labile cysteine residues are oxidized and Keap1 loses its capacity to mediate Nrf2 ubiquitination, stabilizing Nrf2. Nrf2 then accumulates and trans-locates to the nucleus, a process that is enhanced via the phosphorylation of Nrf2 by several protein kinases. The activated Nrf2 then binds to a region of antioxidant response elements (ARE) of numerous antioxidant genes, enhancing their expression. Thus, the Nrf2/ARE pathway is important because it downregulates oxidative stress to restore redox balance, suppresses inflammation (TGF-β and NF-kβ), and enhances xenobiotic metabolism and excretion, apoptosis, and autophagy (**Baird and Yamamoto, 2020**). Moreover, as redox homeostasis is re-established by a Nrf2-induced increase surge in antioxidants and as xenobiotics are metabolized and excreted, ROS signaling terminates. Keap1 then enters the nucleus, binds Nrf2, and transports it back to the cytosol for degradation by proteosomes (**Wakabayashi et al., 2010**). The adaptive functions of Nrf2 signaling have been established in numerous organs with a principal focus on tissues highly involved with antioxidation and detoxification. The cytoprotective roles of Nrf2 have been widely studied in Nrf2-knockout mice, including multiple mouse strains and with tissue-targeted, Nrf2- knockout models (e.g., lung). In these studies, Nrf2 deficiency has been associated with enhancements in the following: organ stress, susceptibility to stressor-induced damage, magnitude of injury, and recovery time (**Zheng et al., 2019; Iizuka et al., 2005; Ishii et al., 2005; Jia et al., 2020a,b**). Prolonged exposures to oxidative stressors, however, may overwhelm the hormetically regulated Nrf2 mechanisms that maintain redox homeostasis and, as such, induce pro-oxidant states that promote proinflammatory reactions. For example, the process of polarizing macrophages from anti-inflammatory (M2) to proinflammatory (M1) states with the treatment of various polarization agents was recently shown to be dependent on the hormetic dose response of the specific polarization agent (**Calabrese et al., 2018**). Macrophage polarization is thus linked to hormesis and furthermore is regulated by increasing levels of mitochondria-generated ROS, which also activate the MAPK and NF-kβ downstream pathways to affect more broadly an inflammatory state, including the subsequent induction of TNFα (**Tan et al., 2016**). Thus, in elevating antioxidant levels (e.g., GSH) to regulate redox balance, Nrf2 indirectly affects whether the immune system is predisposed toward anti-inflammatory or pro-inflammatory states (**Yeang et al., 2012**).

Both anti-inflammatory and pro-inflammatory states are, however, double-edged swords capable of preventing and promoting diseases. Pro-inflammatory states activate the immune system to destroy infectious agents and cancer cells while anti-inflammatory states restrain the immune system to eliminate key etiological factors of chronic inflammation that contribute to many chronic diseases, such as Alzheimer's and Parkinson's diseases, heart disease, diabetes, and cancer (**Tan et al., 2016**). Substantial pathology studies have associated elevated levels of TNFα with inflammatory processes. Blockage of TNFα with antagonists is generally effective in the treatment of inflammatory conditions. Despite this use of TNFα antagonists, they can be problematic when chronically employed, increasing cancer risks as well as demyelination and cardiovascular disorders, suggesting that TNFα acts in a biphasic dose-response manner, displaying

pro- and anti-inflammatory responses as a function of dose (**Shanmugam et al., 2016**). Studies with cardiomyocytes showed that TNFα acts biphasically over a 50-fold concentration range. Within the low concentration range of 2–5 ng/mL, TNFα enhanced nuclear translocation of Nrf2 along with increased binding to the DNA promoter region and the transactivation of the Nrf2 gene. These actions were associated with a 50% increase in intracellular GSH. Higher concentrations of TNFα, however, suppressed the GSH response. Similar biphasic dose-response findings were reported for NQO1, HO-1 and G-6-PD. These findings demonstrate that exposure of TNFα to cardiomyocytes, at concentrations far below those that induced inflammation, affects an increase in Nrf2 and in the subsequent anti-inflammatory response (**Shanmugam et al., 2016**). Such observations are particularly significant since they reveal a biphasic effect of TNFα in the regulation of the redox-sensitive Keap1/Nrf2 antioxidant pathway as well as the involvement of substantial crosstalk between Nrf2 and NF-kB in the process of regulating the anti- and pro-inflammatory states.

Hormesis and Adverse Effects

Low-dose stimulatory effects that conform to the quantitative features of a hormetic dose response have the potential to result in beneficial, adverse, or neutral effects. While there is much focus on the beneficial biomedical and public health implications of hormetic dose responses, there are a considerable number of situations identified in which the low-dose stimulation is undesirable and potentially adverse. In fact, it was reported in the 1940s that low doses of antibiotics such as penicillin enhanced the survival and growth of harmful bacteria while killing these microbes at higher concentrations (**Randall et al., 1947**). This has also been reported for copious examples of antitumor drugs, where they often enhance the proliferation of large numbers of various animal and human tumor cell lines (**Calabrese et al., 2006, 2008, 2010**). Adverse effects that conform to the features of the hormesis dose response may also occur for agents considered as endocrine disruptors (**Calabrese, 2008a**). This is often referred to as a 'nonmonotonic dose-response relationship (NMDR) (See Chapter 1.03 in this volume). For example, if an agent was to stimulate the enlargement of the prostate gland, the hormetic dose–response model would predict increases at maximum in the 30–60% zone and that is precisely what is displayed in the scientific literature. Thus, many effects of endocrine-disrupting agents act hormetically. Despite the fact that many endocrine disruptors act in this manner, the research community in this area rarely uses the term hormesis. The value of the hormetic dose–response term, versus 'nonmonotonic dose-response' is that hormesis refers to a specific type of biphasic dose response, having a definite set of quantitative features and a definable relationship to the traditional toxicological threshold. These features of the hormetic dose response make it extremely useful for the risk assessment process and those more involved hazard assessment testing. It seems that the reluctance to use the term hormesis may be related to earlier but incorrect views that hormetic effects are always beneficial, a position that was convincingly refuted well over a decade ago (**Calabrese and Baldwin, 2002b**).

Hormesis can be Reconciled With the LNT Dose–Response Models for Low-Dose Carcinogen Risk Assessment

Calabrese (2015) has reported that it is possible to integrate the LNT and hormetic dose–response models via a model uncertain methodology that optimizes the public health response. In this case, it was shown that the nadir of the hormetic dose response in animal model studies following standard uncertainty factor (UF) risk assessment methods occurs at the dose that is associated with a 10–4 cancer risk (**Calabrese and Cook, 2005**). Increasing or decreasing the dose from this optimized point will result in an estimated increase in tumor response within the population (**Fig. 7**). In order to adopt this perspective, it would be necessary for supporters of LNT and hormesis to recognize the possibility that their views have the potential to be incorrect and that the opposing views can have the potential to be correct. By doing so, it creates a functional framework for model uncertainty. While it is unlikely that such opposing groups would actually accept these possibilities in an intellectual sense per se, they could be accepted as a practical measure. For example, if LNT was to be correct, the cancer incidence would increase linearly with increasing dose, but in a manner that could not be verified because 1/10,000 is a risk that is normally far below the capacity of epidemiological studies to evaluate. On the other hand, should the hormetic perspective be correct, the reduction in people developing tumors would be markedly profound. The upside benefit for society therefore is sizable and significant if hormesis is correct. The benefit/risk ratio for adopting the integration is clearly in society's best interest, rather than to continue to use the LNT as the default. This approach would still permit the opposing dose–response model camps to continue their research while at the same time recognize that continuing use of the LNT as the default is scientifically policy and public health wise unsustainable. Furthermore, hormetic dose responses can be experimentally validated, but the same cannot be shown for predictions based on the LNT, providing an additional reason for the inclusion of the hormetic perspective within a model uncertainty mode. This issue has been recently addressed with suggestion for better integrating experimental and epidemiologic findings for applications to human risk assessment via improved weight-of-evidence procedures (**Calabrese et al., 2023c**).

Hormesis can be Used for Noncarcinogen Risk Assessment

Integrating hormesis into noncarcinogen risk assessment using the EPA framework assumes the consideration of high-risk groups that are more sensitive than the average person by a 10-fold factor. This is a critical factor in the risk assessment process when using a hormetic framework because the hormetic benefits for the normal and high-risk segments of the population do not occur at the same dose but are

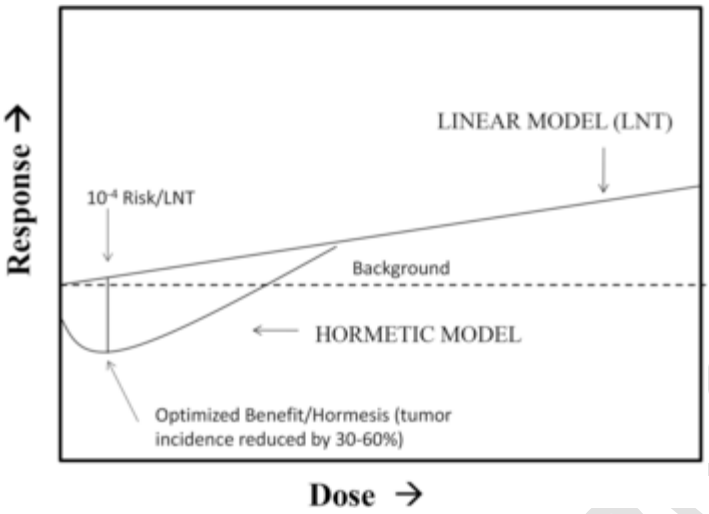


Fig. 7 Integration of hormesis and LNT for risk assessment. Adapted from Calabrese, E.J., Shamoun, D.Y. Hanekamp, J.C., *et al*., 2015b. Cancer risk assessment: Optimizing human health through linear dose-response models. Food and Chemical Toxicology 81, 137–140.

separated by a 10-fold difference in dose. In the current non-hormesis scheme used by regulatory agencies, the goal is to assure that the dose is in a safe zone with an adequate margin of safety via the use of uncertainty factors. This process does not acknowledge the possibility of beneficial responses from low doses of the regulated agent. Using a hormetic framework, beneficial responses for the average person would be expected to occur in the approximate area of a safe dose for the high-risk segment of the population. Benefits for the high-risk segment of the population would be expected to occur at a dose about 10-fold lower (**Calabrese and Cook, 2005**). Thus, the current risk assessment process used by EPA would be expected not only to protect the normal person but also to include some component of hormetic benefits. However, this would not be the case for the high-risk segment that would receive only protection from harm but none of the predicted hormetic benefits. Whether a hormetic effect is beneficial, harmful, or neutral needs to be evaluated within the biological context of the experiment. This places new challenges and opportunities for regulatory agencies for better application of dose response information.

The First use of Hormesis in Regulatory Toxicology

While one may think that the use of hormesis in regulation is simply an issue affecting society at the present time, it might be surprising to learn that the first known use of the hormesis concept in environmental regulation occurred over a century ago (1912) in California. In a public hearing concerning the environmental effects of a major smelter facility (**Calabrese, 2008b; Lipman, 1915**), it was revealed that low levels of arsenic and lead could enhance plant growth, thereby having a potential beneficial effect for some agricultural activities (**Calabrese, 2008b; Haywood, 1905, 1907, 1910; Lipman, 1915; Lipman and Gericke, 1917; Lipman and Wilson, 1913**). This research was funded by the state of California and conducted at the University of California at Berkeley by Professor Charles Lipman, who later become dean at that institution.

How Does the Concept of Tolerance Relate to Hormesis?

While there has been much discussion on preconditioning/adaptive responses and hormesis in this article, there has been surprisingly no broad-based effort to explore such a relationship for the concept of tolerance, with the exception of ethanol. Tolerance is a type of adaptive response following a series of exposures to various agents but often with agents of health concern, such as ethanol, insecticides, and antibiotics. During the acquisition of tolerance, a dose that caused biological responses at low doses will eventually be found not to cause such effects with continuing treatment. At that point, a higher dose is needed to affect the same response in the now tolerant subject. The relationship between hormesis and tolerance has been assessed in detail with respect to ethanol consumption in various mouse strains. More specifically, ethanol exposures consistently induce a low-dose stimulation of locomotion and a higher dose inhibition. In general, however, a tolerance only developed to the higher dose inhibitory effects of the ethanol and not to the stimulatory effects that are induced by the low-dose treatments (**Crabbe et al., 1982; Erwin et al., 1992; Masur et al., 1986; Tabakoff and Kiianmaa, 1982**). Based on these findings, the hormesis response to ethanol-induced stimulation of locomotion was independent of tolerance development.

The concepts of hormesis and tolerance are of such basic and applied interest that further research on how these concepts interact would be important.

Hormesis and Longevity: A Contrast Between Pharmacology and Regulatory Toxicology

The modern regulatory history of toxicology has been driven by a focus to prevent adverse effects. In the case of life span, this may be seen in a desire not to permit a dosage of a toxic agent to reduce life span. While this is a critical public health goal, the field of biogerontology has published hundreds of studies in which low doses of stressor agents such as thermal, oxidative, ionizing radiation, and chemical agents may increase longevity in multiple species via the process of hormesis (**Calabrese et al., 2023a**). There is no reason why these life-serving perspectives should not be appropriately integrated into public health promoting programs. However, the EPA and other regulatory agencies typically do not include any consideration of such potential positive effects.

Triphasic Dose–Response Relationships

This article concerns the occurrence, generality, mechanisms, and biological and public health significance of hormetic biphasic dose–response relationships. It has contrasted such biphasic dose responses with the linear and threshold dose–response models. However, it is important to point out that the range of dose–response models may be broader, including the possibility of triphasic dose responses, and even more complex relationships. While identification of biphasic dose responses require far more experimental resources than typical studies using a few high doses and the extrapolation approach of modern regulatory agencies, triphasic dose responses would add another level of complexity and resource allocation. Nonetheless, evidence has emerged that triphasic dose responses may occur for important endpoints and may be critical in understanding how biological systems act at low doses of stressor agents. For example, ionizing radiation induces mutations at high doses that exceed the capacity for complete repair (**Sykes and Day, 2007**). However, at lower doses of ionizing radiation, repair processes can be sufficiently upregulated such that they not only efficiently repair the newly induced damage but also reduce background damage, yielding a net lower mutational rate than the unexposed controls. This would appear as a J-shaped hormetic dose–response relationship. However, at even lower doses, there may be an increase in mutational damage that is not repaired. The unrepaired damage at very low doses may be due to the possibility that the effects induced are too small to be discerned by damage detection systems, thereby failing to induce the adaptive/reparative system. Combining each of this dose perspective results in a triphasic dose–response relationship. In such systems, a triphasic dose response may best account for the findings. To what extent triphasic dose responses exist would be important to determine along with their potential evolutionary, biological, and public health significance.

Hormesis Principles and Clinical/Risk Assessment Integration

Tables 2 and 3 provide a broad integration of the experimental findings that form the bases of the hormetic principles. **Table 4** provides a summary of the toxicological/risk implications of hormesis and its potential significance to the pharmaceutical industry for drug evaluation.

Chapter in Perspective

This article on hormetic dose responses was intended to provide the reader with a historical perspective on the origin, acceptance, evolution, validation, and utility of the major dose responses, such as the threshold and LNT models and how they competed with hormesis and each other within toxicology, pharmacology, and risk assessment communities. The article argues that the toxicology, biomedical, and regulatory communities made an error of historical proportions when they intentionally rejected the hormetic biphasic dose–response model because its founder, Hugo Schulz, claimed that it provided the explanatory principle of the medical practice of homeopathy. The actions of the biomedical and toxicology communities to suppress and marginalize the hormetic dose response have largely continued down to the present time (**Calabrese, 2011**). It is important to understand that the scientific community tried to kill an idea (i.e., hormesis) due to a "political" battle between two entities: traditional medicine and homeopathy. The suppression of the hormesis concept was collateral damage in this medical and economic conflict. However, recent generations of toxicologists have not been aware of these historical determinants that have guided (or misguided) our field for decades and generations. Such dose–response-based failings and prejudices have undermined research developments, new discoveries, risk assessment procedures, and how to properly design studies with respect to dose number and dose spacing, among others. These prejudices still continue to affect regulatory toxicology, with its long-standing emphasis on high dose and few doses, with its failure to consider the entire dose–response continuum and its striking inability to consider the concept of adaptive/beneficial responses in the risk assessment process. It is hoped that the article will play a role in both educating and reorienting the next generation of toxicologists to study the entire dose–response continuum and to be open to the realistic possibilities that cells and organs can respond very differently to toxic agents at low- versus high-dose and at low- versus high-dose rates. Failure to incorporate this intellectual framework into the education of future toxicologists will continue to impede the capacity of the field to address the

Table 4 Implications of hormesis for toxicology/risk assessment and clinical practices/biomedical/pharmaceutical companies.

Toxicology/risk assessment
Changes strategy for hazard assessment, altering animal model selection, range of endpoints measured, and study design, affecting the number of doses, dose range, and statistical power
Alters biostatistical modeling to estimate responses below control background disease incidence
Differentiates dose optima (i.e., benefits) for normal- and high-risk segments of the population
Creates an evaluative framework to assess benefits or harm below the traditional toxicological threshold
Creates a new framework for quantitatively altering the magnitude of uncertainty factors in the risk assessment process
Creates a functional framework to integrate LNT and hormesis within a model uncertainty framework
Clinical practices/pharmaceutical companies
Drug performance expectations will be constrained by the limits of plasticity, which is described by the quantitative features of the hormetic dose response
Drugs that are designed to act at high doses may have hormetic effects at low doses, with possible undesirable effects (e.g., antitumor agents, antibiotics, chemical disinfectants)
Modification of biological set points will be constrained by the quantitative features of the hormetic dose response
Clinical trials need to recognize interindividual variation for hormetic responses

Adapted from Calabrese, E.J., 2008a. Hormesis: Why it is important to toxicology and toxicologists. Environmental Toxicology and Chemistry 27, 1451–1474.

most critical issues that confront the field, that is, knowing the nature of the dose response in the low-dose zone and that figuring out what that means for the environment and people. Continuing to operate in a toxicology world that is dominated by high doses and high-dose rates will lessen the significance of the discipline at a time that society should be guided by it. Hopefully, the article will provide a "high" dose of intellectual smelling salts that will lead to a clearer research and regulatory vision for low-dose toxicology.

Acknowledgment

This research has been supported by awards from the US Air Force and ExxonMobil Foundation. The U.S. Government is authorized to reproduce and distribute for governmental purposes notwithstanding any copyright notation thereon. The views and conclusions contained herein are those of the author and should not be interpreted as necessarily representing policies or endorsement, either expressed or implied. Sponsors had no involvement in study design, collection, analysis, interpretation, writing and decision to and where to submit for publication consideration.

Uncited References

Bodar et al., 1988; **Bors and Zimmr, 1970**; **Bottino and Sparrow, 1973**; **Calabrese, 2013**; **Cookson et al., 1995**; **Daston et al., 1991**; **Doyle et al., 1975**; **Folker-Hansen et al., 1996**; **Fulder, 1977**; **Hegab et al., 2005**; **Hodjat, 1971**; **Hunter and Krithayakiern, 1971**; **Laughlin et al., 1989**; **Lee et al., 1985**; **Levings, 1977**; **McIntyre, 1973**; **Meredith et al., 1977**; **Miller et al., 1945**; **Monia et al., 1987**; **Nayak et al., 1996**; **Nutman and Roberts, 1962**; **Pahan et al., 1993**; **Rahn, 1915**; **Sukata et al., 2002**; **US National Academy of Sciences (US NAS), 1977**; **Varlinskaya et al., 2001**.

References

OSHA (1980) (Occupational Safety and Health Administration) In: Employment Safety and Health Guide. OSHA Rules on the Identification, Classification an Regulation of Potential Occupational Carcinogens, Vol. 454. pp. 5002–5296, Commerce Clearing House, Inc.,, Washington DC.

Abete, P., Testa, G., and Galizia, G., et al. (2005) Tandem action of exercise training and food restriction completely preserves ischemic preconditioning in the aging heart. *Experimental Gerontology* 40: 43–50.

Agathokleous, E., Brown, P.H., and Calabrese, E.J. (2021a) A gift from parent to offspring: Transgenerational hormesis. *Trends in Plant Science* 26: 1098–1100.

Agathokleous, E., Guedes, R.N.C., Calabrese, E.J., Fotopoulos, V., and Azevedo, R.A. (2021b) Transgenerational hormesis: What do parents sacrifice for their offspring? *Current Opinion in Environmental Science & Health* 29: 100380.

Ahmed, S.M., Luo, L., Namani, A., Wang, X.J., and Tang, X. (2016) Nrf2 signaling pathway: pivotal roles inflammation. *Biochimica et Biophysica Acta Molecular Basis of Disease* 1863: 585–597.

Ancel, P. and Lallemand, S. (1928) Sur la protection contre l'action des rayons X par une irradiation prealable (radiophylaxie). *Comptes Rendus Societe de Biologie* 99: 1588–1590.

Arrhenius, S. (1915) *Quantitative Laws in Biological Chemistry*. Bell & Sons Ltd., London.

Baird, L. and Yamamoto, M. (2020) The molecular mechanisms regulating the KEAP1-NRF2 Pathway. *Molecular and Cellular Biology* 40 (13) e00099-20.

Beckon, W., Parkins, C., Maximovich, A., and Beckon, A.V. (2008) A general approach to modeling biphasic relationships. *Envrionmental Science & Technology* 45: 1308–1314.
Belz, R.G. (2020) Trans-generational impacts of paternal irradiation in a cricket: Damage, life-history features and hormesis in F1 offspring. *Pest Management Science* 76: 3056–3065.
Belz, R.G. and Piepho, H.-P. (2012) Modeling effective dosages in hormetic dose-response studies. *PLOS One* 7: e33432.
Berkson, J. (1944) Application of the logistic function to bio-assay. *Journal of the American Statistical Association* 39: 357–365.
Berkson, J. (1951) Why I prefer logics to probate. *Biometrics* 7: 329–339.
Blau, M. and Altenburger, K. (1922) Uber einige Wirkungen von Strahlen. II. *Zeitschrift für Physik* 12: 315–329.
Bliss, C.I. (1935a) Estimating the dosage-mortality curve. *Journal of Economic Entomology* 25: 646–647.
Bliss, C.I. (1935b) The calculation of the dosage-mortality curve. *Annals of Applied Biology* 22: 134–167.
Bliss, C.I. (1935c) The comparison of dosage-mortality data. *Annals of Applied Biology* 22: 307–333.
Bliss, C.I. (1940) The relation between exposure time, concentration and toxicity in experiments on insecticides. *Annals of the Entomological Society of America* 33: 721–766.
Bliss, C.I. (1941) Biometry in the service of biological assay. *Industrial Engineering and Chemistry* 13: 84–88.
Bliss, C.I. (1956) The calculation of microbial assays. *Bacteriological Reviews* 20: 243–258.
Bliss, C.I. (1957) Some principles of bioassay. *American Scientist* 45: 449–466.
Bliss, C.I. and Packard, C. (1941) Stability of the standard dosage-effect curve for radiation. *American Journal of Roentgenology and Radium Therapy* 46: 400–404.
Bliss, C.I. and Cattell, M. (1943) Biological assay. *Annual Review of Physiology* 5: 479–539.
Bliss, C.T. (1939) The toxicity of poisons applied jointly. *Annals of Applied Biology* 26: 585–615.
Bloedau, C.V. (1884) *General Medical Central* 93: 1362.
Bodar, C.W.M., Van Leeuwen, C.J., Voogt, P.A., and Zandee, D.I. (1988) Effect of cadmium on the reproduction strategy of *Daphnia magna*. *Aquatic Toxicology* 12: 301–310.
Bohme, H., 1986. Hugo Schulz (8/6/1853–7/13/1932). His life and work. Ph.D. Thesis, Freien University of Berlin, Berlin, Germany.
Bors, J. and Zimmr, K. (1970) Effects of low doses of x-rays on rooting and yield of carnation. *Stimulation Newslette* 1: 16–21.
Bottino, P.F. and Sparrow, A.H. (1973) The influence of seasonal variation on survival and yield of lettuce irradiated with constant rate, fallout decay or buildup and fallout decay simulation treatments. *Radiation Botany* 13: 27–36.
Branham, S.E. (1929) The effects of certain chemical compounds upon the course of gas production by Baker's yeast. *Journal of Bacteriology* 18: 247–284.
Brooks, S.C. (1918) A theory of the mechanism of disinfection, hemolysis and similar processes. *Journal of General Physiology* 1: 61–80.
Bruce, R.D., Carlton, W.W., and Ferber, K.H., et al. (1981) Reexamination of the ED01 study: Why the society of toxicology became involved. *Fundamental and Applied Toxicology* 1: 26–128.
Bryan, W.R. and Shimkin, M.B. (1940) Quantitative analysis of dose-response data obtained with carcinogenic hydrocarbons. *Journal of the National Cancer Institute* 1: 807–833.
Buchanan, R.E. and Fulmer, E.I. (1930) *Physiology and Biochemistry of Bacteria. Effects of Environment Upon Microorganisms*. The Williams and Wilkins Co.,, Baltimore, MD.
Calabrese, E.J. (1990) *Multiple Chemical Interactions*. CRC Press,, Boca Raton, FL.
Calabrese, E.J. (2005) Historical blunders: how toxicology got the dose-response relationship half right. *Cellular and Molecular Biology* 51: 643–654.
Calabrese, E.J. (2008a) Hormesis: why it is important to toxicology and toxicologists. *Environmental Toxicology and Chemistry* 27: 1451–1474.
Calabrese, E.J. (2008b) Another California milestone: The first application of hormesis in litigation and regulation. *International Journal of Toxicology* 27: 31–33.
Calabrese, E.J. (2009) Getting the dose-response wrong: Why hormesis became marginalized and the threshold model accepted. *Archives of Toxicology* 83: 227–247.
Calabrese, E.J. (2010) Hormesis is central to toxicology, pharmacology and risk assessment. *Human and Experimental Toxicology* 29: 249–261.
Calabrese, E.J. (2011) Toxicology rewrites its history and rethinks its future: Giving equal focus to both harmful and beneficial effects. *Environmental Toxicology and Chemistry* 30: 2658–2673.
Calabrese, E.J. (2013) Origin of the linearity no threshold (LNT) dose-response concept. *Archives of Toxicology* 87: 1621–1633.
Calabrese, E.J. (2013a) Hormetic mechanisms. *Critical Reviews in Toxicology* 43: 580–606.
Calabrese, E.J. (2013b) Biphasic dose responses in biology, toxicology and medicine: Accounting for their generalizability and quantitative features. *Environmental Pollution* 182: 452–460.
Calabrese, E.J. (2015) On the origins of the linear no-threshold (LNT) dogma by means of untruths, artful dodges and blind faith. *Environmental Research* 142: 432–442.
Calabrese, E.J. (2016a) Preconditioning is hormesis. Part I. Documentation, dose-response features and mechanistic foundations. *Pharmacological Research* 110: 242–264.
Calabrese, E.J. (2016b) Preconditioning is hormesis. Part II. How the conditioning dose mediates protections: dose optimization within temporal and mechanistic frameworks. *Pharmacological Research* 110: 265–275.
Calabrese, E.J. (2016c) Pre-and post-conditioning hormesis in elderly mice, rats and humans: Its loss and restoration. *Biogerontology* 17: 681–702.
Calabrese, E.J. and Baldwin, L.A. (1992) Lead-induced cell-proliferation and organ-specific tumorigenicity. *Drug Metabolism Review* 24: 409–416.
Calabrese, E.J. and Baldwin, L.A. (2000a) Chemical hormesis: its historical foundations as a biological hypothesis. *Human and Experimental Toxicology* 19: 2–31.
Calabrese, E.J. and Baldwin, L.A. (2000b) The marginalization of hormesis. *Human and Experimental Toxicology* 19: 32–40.
Calabrese, E.J. and Baldwin, L.A. (2000c) Radiation hormesis: its historical foundations as a biological hypothesis. *Human and Experimental Toxicology* 19: 41–75.
Calabrese, E.J. and Baldwin, L.A. (2000d) Radiation hormesis: the demise of a legitimate hypothesis. *Human and Experimental Toxicology* 19: 76–84.
Calabrese, E.J. and Baldwin, L.A. (2000e) Tales of two similar hypotheses: the risk and fall of chemical and radiation hormesis. *Human and Experimental Toxicology* 19: 85–97.
Calabrese, E.J. and Baldwin, L.A. (2001) U-shaped dose-responses in biology, toxicology, and public health. *Annual Reviews of Public Health* 22: 15–33.
Calabrese, E.J. and Baldwin, L.A. (2002a) Hormesis and high-risk groups. *Regulatory Toxicology and Pharmacology* 35: 414–428.
Calabrese, E.J. and Baldwin, L.A. (2002b) Applications of hormesis in toxicology, risk assessment and chemotherapeutics. *Trends in Pharmacological Sciences* 23: 331–337.
Calabrese, E.J. and Baldwin, L.A. (2003) The hormetic dose-response model is more common than the threshold model in toxicology. *Toxicological Sciences* 71: 246–250.
Calabrese, E.J. and Cook, R.R. (2005) Hormesis: How it could affect the risk assessment process. *Human and Experimental Toxicology* 24: 265–270.
Calabrese, E.J. and Blain, R. (2005) The occurrence of hormetic dose responses in the toxicological literature, the hormesis database: an overview.

Toxicology and Applied Pharmacology 202: 289–301.
Calabrese, E.J. and Blain, R.B. (2009) Hormesis and plant biology. *Environmental Pollution* 157: 42–48.
Calabrese, E.J. and Blain, R.B. (2011) The hormesis database: the occurrence of hormetic dose responses in the toxicological literature. *Regulatory Toxicology and Pharmacology* 61: 73–81.
Calabrese, E.J. and Mattson, M.P. (2011) Hormesis provides a generalized quantitative estimate of biological plasticity. *Journal of Cell Communication and Signaling* 5: 25–38.
Calabrese, E.J. and Calabrese, V. (2013a) Reduction of arthritic symptoms by low dose radiation therapy (LD-RT) is associated with an anti-inflammatory phenotype. *International Journal of Radiation Biology* 89: 278–286.
Calabrese, E.J. and Calabrese, V. (2013b) Low dose radiation therapy (LD-RT) is effective in the treatment of arthritis: Animal model findings. *International Journal of Radiation Biology* 89: 287–294.
Calabrese, E.J. and Giordano, J. (2021) Ultra low doses and biological amplification: Approaching Avogadro's number. *Pharmacological Research* 170: 105738.
Calabrese, E.J., Iavicoli, I., and Calabrese, V. (2013) Hormesis: Its impact on medicine and health. *Human and Experimental Toxicology* 32: 120–152.
Calabrese, E.J., Shamoun, D.Y., and Hanekamp, J.C. (2015b) Cancer risk assessment: Optimizing human health through linear dose-response models. *Food and Chemical Toxicology* 81: 137–140.
Calabrese, E.J., Calabrese, V., and Giordano, J. (2017) Role of hormesis in functional performance and protection of neural systems. *Brain Circulation* 3: 1–13.
Calabrese, E.J., Staudenmayer, J.W., Stanek, E.J., III, and Hoffmann, G.R. (2006) Hormesis outperforms threshold model in National Cancer Institute antitumor drug screening database. *Toxicological Sciences* 94: 368–378.
Calabrese, E.J., Stanek, E.J., III, Nascarella, M.A., and Hoffmann, G.R. (2008) Hormesis predicts low-dose-responses better than threshold models. *International Journal of Toxicology* 27: 369–378.
Calabrese, E.J., Hoffmann, G.R., Stanek, E.J., III, and Nascarella, M.A. (2010) Hormesis in high-throughput screening of antibacterial compounds in *E. coli*. *Human and Experimental Toxicology* 29: 667–677.
Calabrese, E.J., Agathokleous, E., Kozumbo, W.J., and Leonard, D.L. (2019) Estimating the range of the maximum hormetic stimulator response. *Environmental Research* 170: 337–343.
Calabrese, E.J., Dhawan, G., Kapoor, R., Iavicoli, I., and Calabrese, V. (2015a) What is hormesis and its relevance to healthy aging and longevity? *Biogerontology* 16: 693–707.
Calabrese, E.J., Giordano, J.J., Kozumbo, W.J., Leak, R.K., and Bhatia, T.N. (2018) Hormesis mediates dose-sensitive shifts in macrophage activation patterns. *Pharmacological Research* 137: 236–249.
Calabrese, E.J., Pressman, P., and Hayes, A.W., et al. (2023a) Hormesis determines lifespan. *Ageing Research Reviews*.
Calabrese, E.J., Osakabe, N., and Di Paola, R., et al. (2023b) Hormesis defines the limits of lifespan. *Ageing Research Reviews* 91: 102071.
Calabrese, E.J., Pressman, P., and Hayes, A.W., et al. (2023c) Hormesis, biological plasticity, and implications for clinical trial research. *Ageing Research Reviews* 90: 102028.
Carlisle, S.M., Burchart, P.A., and Mitchel, R.E.J. (2010) Cancer and non-cancer risks in normal and cancer-prone Trp53 heterozygous mice exposed to high-dose radiation. *Radiation Research* 173: 40–48.
Chick, H. (1908) An investigation of the laws of disinfection. *Journal of Hygiene* 8: 92–158.
Civciristov, S., Ellisdon, A.M., and Suderman, R., et al. (2018) Preassembled GPCR signaling complexes mediate distinct cellular responses to ultralow ligand concentrations. *Science Signal* 11 eaan1188.
Clark, A.J. (1937) *Handbook of Experimental Pharmacology*. Verlag Von Julius Springer,, Berlin.
Clifton, C.E. (1957) *Death of bacteria. Introduction to Bacterial Physiology*. McGraw-Hill Book Company Inc,, New York.
Cookson, M.R., Mead, C., Austwick, S.M., and Pentreath, V.W. (1995) Use of the MTT assay for estimating toxicity in primary astrocyte and C6 glioma cell cultures. *Toxicology in Vitro* 9: 39–48.
Coulter, H.L. (1972) *Homeopathic Medicine*. Formur,, St. Louis, MO.
Coulter, H.L. (1982) *BT Divided Legacy: The Conflict Between Homeopathy and the American Medical Association*. North Atlantic Books, Richmond, CA.
Crabbe, J.C., Johnson, N., Gray, D., Kosobud, A., and Young, E.R. (1982) Biphasic effects of ethanol on open-field activity: Sensitivity and tolerance in C57/6N and DBA/2N mice. *Journal of Comparative and Physiological Psychology* 96: 440–451.
Crowther, J.A. (1926) The action of x-rays on *Colpidium colpoda*. *Proceedings of the Royal Society of London B* 100: 396.
Csaba, G., Kovacs, P., and Pallinger, E. (2007) How does the unicellular Tetrahymena utilise the hormones that it produces? Paying a visit to the realm of atto-and zeptomolar concentrations. *Cell Tissue Research* 327: 199–203.
Dambrauskas, T. and Cornish, H.H. (1970) Effect of pretreatment of rats with carbon tetrachloride on tolerance development. *Toxicology and Applied Pharmacology* 17: 83–97.
Daston, G.P., Rogers, J.M., and Vesteeg, D.J., et al. (1991) Interspecies comparisons of A/D ratios – A/D ratios are not constant across species. *Fundamental and Applied Toxicolog* 17: 696–722.
Dharmani, M., Mustafa, M.R., Achike, F.I., and Sim, M.K. (2005) Effect of des-aspartate-angiotensin I on the actions of angiotensin II in the isolated renal and mesenteric vasculature of hypertensive and STZ-induced diabetic rats. *Regulation Peptides* 129: 213–219.
Doyle, J.J., Marshall, R.T., and Pfander, W.H. (1975) Effects of cadmium on the growth and uptake of cadmium by microorganisms. *Applied Microbiology* 29: 562–564.
Eijkman, C. (1908) Die Ueberlebungskurve bei Abstotung der Bakterien durch Hitze. *Biochemische Zeitschrift* 11: 12–20.
Emmens, C.W. (1940/1941) The dose/response relation for certain principles of the pituitary gland, and of the serum and urine of pregnancy. *Journal of Endocrinology* 2: 194–225.
Ershova, E.S., Sergeeva, V.A., and Tabakov, V.J., et al. (2016) Functionalized fullerene increases NF-kB activity and blocks genotoxic effect of oxidative stress in serum-starving human embryo lung diploid fibroblasts. *Oxidative Medicine and Cellular Longevity* 2016: 9895245.
Erwin, V.G., Radcliffe, R.A., and Jones, B.C. (1992) Chronic ethanol-consumption produces genotype-dependent tolerance to ethanol in LS/IBG and SS/IBG mice. *Pharmacology Biochemistry and Behavior* 41: 275–281.
Ewald, K.A. and Calabrese, E.J. (2001) Lead reduces the nephrotoxicity of mercuric chloride. *Ecotoxicology and Environmental Safety* 48: 215–218.
Finney, D.J. (1943/1944) The application of the probit method to toxicity test data adjusted for mortality in the controls. *Annals of Applied Biology* 30: 68–74.
Finney, D.J. (1949) The choice of a response metameter in bio-assay. *Biometrics* 5: 261–272.
Finney, D.J. (1952) *Probit Analysis, A Statistical Treatment of the Sigmoid Response Curve*, 2nd ed. Cambridge University Press,, London.
Flood, J.F., Smith, G.E., and Cherkin, A. (1983) Memory retention—potentiation of cholinergic drug-combination in mice. *Neurobiology of Aging* 4: 37–43.
Flood, J.F., Smith, G.E., and Cherkin, A. (1985) Memory enhancement—supra-additive effect of subcutaneous cholinergic drug-combinations in mice. *Psychopharmacology* 86: 61–67.
Folker-Hansen, P., Krogh, P.H., and Holmstrup, M. (1996) Effect of dimethoate on body growth of representatives of the soil living mesofauna.

Ecotoxicology and Environmental Safety 33: 207–216.
Fuciarelli, T.M. and Rollo, C.D. (2020) Trans-generational impacts of paternal irradiation in a cricket: Damage, life-history features and hormesis in F1 offspring. *Dose Response* 20: 1–8.
Fulder, S.J. (1977) The growth of cultured human fibroblasts treated with hydrocortisone and extracts of the medicinal plant *Panax ginseng*. *Experimental Gerontology* 12: 125–131.
Gaddum, J.H. (1945) Lognormal distributions. *Nature* 156: 463–466.
Gaddum, J.H. (1953) Bioassays and mathematics. *Pharmacological Reviews* 5: 87–134.
Glende, E.A., Jr. (1972) Carbon tetrachloride-induced protection against carbon tetrachloride toxicity. The role of liver microsomal drug-metabolizing system. *Biochemical Pharmacology* 21: 1697–1702.
Gottbrecht, C., 1886. Experimentalle Untersucheunge Dissertation, Greifswald, Germany.
Greaves, J.E. (1913a) The influence of arsenic upon the biological transformation of nitrogen in soils. *Biochemical Bulletin* 3: 2–16.
Greaves, J.E. (1913b) Stimulating influence of arsenic upon the nitrogen-fixing organisms of the soil. *Journal of Agricultural Research* 6: 389–416.
Greaves, J.E. and Carter, E.G. (1924) Influence of sodium arsenite on microflora of soil. *Botanical Gazette* 77: 63–72.
Gurevich, K.G. (2001) Low doses of biologically active substances: Effects, possible mechanisms, and features. *Cell Biology International* 25: 475–484.
Haywood, J.K. (1905) Injury to vegetation by smelter fumes. *USDA Bureau of Chemistry, Bulletin* 89: 1–23.
Haywood, J.K. (1907) Injury to vegetation and animal life by smelter fumes. *USDA Bureau of Chemistry, Bulletin,* 133: 998–1009.
Haywood, J.K. (1910) Injury to vegetation and animal life by smelter fumes. *USDA Bureau of Chemistry, Bulletin* 113: 1–63 (revised).
Hegab, A.E., Hosoya, T., and Nomura, A., et al. (2005) Transcription factor Nrf2 plays a pivotal role in protection against elastase-induced pulmonary inflammation and emphysema. *Journal of Immunology* 175: 6968–6975.
Hewlett, R.T. (1909) The Milroy Lectures of disinfection and disinfectants. *The Lancet* 13: 20–27.
Hodjat, S.H. (1971) Effects of sublethal doses of insecticides and of diet and crowding of *Dysdercus fasciatus* sign. (Hem., Pyrrhocoridae). *Bulletin of Entomological Research* 60: 367–378.
Hoffman, G., 1884. Experimentalle Untersuchungen uber die Wirkung der Aneisensaure. Inaugural Disseration, Greifswald, Germany.
Hofmann, P., 1922. Ueber die Gultigkeit des Arndt-Schulzschen biologischen Grundgesetzes bei der Wirkung von Bakteriengiften. Inaugural Dissertation, tierarztl. Fakultat. Munchen (Translated from the German by University of Massachusetts translation service).
Holweek, F. and Lacassagne, A. (1930) Sur le mecanisme de l'action cytogaustique des radiations. *Comptes Rendus Societe Biologie de Paris* 103: 766–768.
Hotchkiss, M. (1923a) Studies on salt action VI. The stimulating and inhibitive effect of certain cations upon bacteria growth. *Journal of Bacteriology* 8: 141–162.
Hotchkiss, M., 1923b. The influence of various salts upon the growth of bacterium communis. Doctoral Dissertation, Yale University.
Hueppe, F. (1896) In: Jordon, E.O. (ed.), *Principles of Bacteriology*. The Open Court Publishing Company, Chicago.
Hunter, P.E. and Krithayakiern, V. (1971) Effect of gamma radiation upon life expectancy and reproduction in the house cricket, *Acheta domesticus* (Orthoptera: Gryllidae). *Annals of the Entomological Society of America* 64: 119–123.
Iavicoli, I., Carelli, G., Stanek, E.J., III, Castellino, N., and Calabrese, E.J. (2003) Effects of low doses of dietary lead on red blood cell production in male and female mice. *Toxicology Letters* 137: 193–199.
Iizuka, T., Ishii, Y., and Itoh, K., et al. (2005) Nrf2-deficient mice are highly susceptible to cigarette smoke-induced emphysema. *Genes Cells* 10: 1113–1125.
Ishii, Y., Itoh, K., and Morishima, Y., et al. (2005) Transcription factor Nrf2 plays a pivotal role in protection against elastase-induced pulmonary inflammation and emphysema. *Journal of Immunology* 175: 6968–6975.
Ito, T. and Sawauchi, K. (1966) Inhibitory effects on cadmium-induced testicular damage by pretreatment with smaller cadmium dose. *Okajimas Folia Antomica Japonica* 42: 107–117.
Jia, G., Yu, S., and Sun, W., et al. (2020a) Hydrogen sulfide attenuates particulate matter-induced emphysema and airway inflammation through Nrf2-dependent manner. *Frontiers in Pharmacology* 11: 29.
Jia, L., Xiong, Y., Zhang, W., Ma, X., and Xu, X. (2020b) Metformin promotes osteogenic differentiation and protects against oxidative stress-induced damage in periodontal ligament stem cells via activation of the Akt/Nrf2 signaling pathway. *Experimental Cell Research* 386: 111717.
Jones, A., 2010. Knowledge and attitudes concerning hormesis by toxicologists and risk assessors. Ph.D. Thesis, University of Massachusetts, Amherst, MA, USA.
Josephs, I. (1931) In: Guido, L. (ed.), Holzknecht. Radiology, 17. pp. 1316–1318 (Professor Doctor).
Koch, R. (1881) Ueber desinfection. *Mittheil. a. d. kaiserl. Gesundheitsamte* 1: 234–282.
Koller, L.D., Roan, J.G., and Kerkvliet, N.I. (1979) Mitogen stimulation of lymphocytes in CBA mice exposed to lead and cadmium. *Environmental Research* 19: 177–188.
Kronig, B. and Paul, T. (1897) Die chemischen Grudlagen der Lehre von der Ciftwirkung und Disinfection. *Zeitschrift für Hygiene und Infektionskrankheiten* 25: 1–112.
Lamanna, C. and Mallette, M.F. (1965) *Basic Bacteriology its Biological and Chemical Background*, 3rd ed. The Williams & Wilkins Company,, Baltimore, MD.
Laughlin, R.B., Gustafson, R.G., and Pendoley, P. (1989) Acute toxicity of tributyltin (TBT) to early life history stages of the hard shell clam, *Mercenaria mercenaria*. *Bulletin of Environmental Contamination and Toxicology* 42: 352–358.
Lee, T.-C., Oshimura, M., and Barrett, J.C. (1985) Comparison of arsenic-induced cell transformation, cytotoxicity, mutation and cytogenetic effects in Syrian hamster embryo cells in culture. *Carcinogenesis* 6: 1421–1426.
Levings, M.K. (1977) Effects of cadmium chloride on growth and pigments in *Glycine max L.*, *Quercus rubra L.*, *Acer saccharinum L.*, and *Cucumis sativus L.* Master of Science thesis. *Department of Forestry and Natural Resources*. p. 73, Purdue University,, West Lafayette, Indiana.
Lipman, C.B. (1915) Letter to Selby Smelter Commission, San Francisco, CA. In: Holmes, J.A., Franklin, E.C., Gould, R.A. (eds.), *Report of the Selby Smelter Commission. Department of the Interior, Bureau of Mines, Bulletin 98*. p. 61, Washington Government Printing Office, Washington, DC.
Lipman, C.B. and Wilson, F.H. (1913) Toxic inorganic salts and acids as affecting plant growth. *Botanical Gazette* 55: 409–420.
Lipman, C.B. and Gericke, W.F. (1917) Experiments on the effects of constituents of solid smelter wastes on barley grown in pot cultures. *University of California Publications in Agricultural Sciences* 1: 495–587.
Loeb, J. and Northrop, J.H. (1917) On the influence of food and temperature upon the duration of life. *Journal of Biological Chemistry* 32: 103–121.
Luckey, T.D. (1980) *Hormesis With Ionizing Radiation*. CRC Press,, Boca Raton, FL.
Luckey, T.D. (1991) *Radiation Hormesis*. CRC Press,, Boca Raton, FL.
Madsen, T. and Nyman, M. (1907) Zur Theories der Desinfektion. I. *Zeitschrift für Hygiene und Infektionskrankheiten* 57: 388–404.
Mantel, N. and Bryan, W.R. (1961) 'Safety' testing of carcinogenic agents. *Journal of the National Cancer Institute* 27: 455–470.
Marshall, M.S. and Hrenoff, A.K. (1937) Bacteriostasis. *Journal of Infectious Disease* 61: 42–54.
Martel, J., Wu, C.Y., and Peng, H.H., et al. (2020) Plant and fungal products that extend lifespan in *Caenorhabditis elegans*. *Microbiol Cell* 10: 255–269.
Masur, J., Desouza, M.L.O., and Zwicker, A.P. (1986) The excitatory effect of ethanol – absence in rats, no tolerance and increased sensitivity in mice.

Pharmacology Biochemistry and Behavior 24: 1225–1228.
McIntyre, J.D. (1973) Toxicity of methyl mercury for steelhead trout sperm. *Bulletin of Environmental Contamination and Toxicology* 9: 98–99.
Meng, R., Asmaro, K., and Meng, L., et al. (2012) Upper limb ischemic preconditioning prevents recurrent stroke in intracranial arterial stenosis. *Neurology* 79: 1853–1861.
Meng, R., Ding, Y., and Asmaro, K., et al. (2015) Ischemic conditioning is safe and effective for octo-and nonagenarians in stroke prevention and treatment. *Neurotherapeutics* 12: 667–677.
Meredith, P.A., Moore, M.R., and Goldberg, A. (1977) Effect of aluminum, lead and zinc on δ-aminolaevulinic acid dehydratase. *Enzyme* 22: 22–27.
Miller, W.S., Green, C.A., and Kitchen, H. (1945) Biphasic action of penicillin and other sulphonamide similarity. *Nature* 155: 210–211.
Monia, B.P., Butt, T.R., and Mirabelli, C.K., et al. (1987) Induction of metallothionein is correlated with resistance to auranofin, a gold compound, in Chinese hamster ovary cells. *Molecular Pharmacology* 31: 21–26.
Motohashi, H. and Yamamoto, M. (2004) Nrf2-Keap1 defines a physiologically important stress response mechanisms. *Trends in Molecular Medicine* 10: 549–557.
Muller, H.J. (1928) The problem of genic modification. Verhandlungen des V. Internationalen Kongresses fur Vererbungswissenschaft (Berlin, 1927). *Zeitschrift fur inductive abstammungs- und Vererbungslehre. Suppl Band* Vol. 1: 234–260.
Murado, M.A., Vázquez, J.A., Rial, D., and Beiras, R. (2011) Dose-response modelling with two agents: application to the bioassay of oil and shoreline cleaning agents. *Journal of Hazardous Materials* 185: 807–817.
Nayak, S., Mohanty, R.C., and Mohanty, L. (1996) Growth rate of *Ankistrodesmus falcatus* and *Scenedesmus bijuga* in mixed culture exposed to monocrotophos. *Bulletin of Environmental Contamination and Toxicology* 57: 473–479.
Niethammer, A. (1927) The stimulation effect of toxins on fungus and the Arndt-Schulz axion. *Biochemische Zeitschrift* 184: 370–382.
Nutman, F.J. and Roberts, F.M. (1962) Stimulation of two pathogenic fungi by high dilution of fungicides. *Transactions of the British Mycological Society* 45: 449–456.
Offen, D., Sherki, Y., and Melamed, E., et al. (2000) Vasocative intestinal peptide (VIP) prevents neurotoxicity in neuronal cultures: Relevance to neuroprotection in Parkinson's disease. *Brain Research* 854: 257–262.
Packard, C. (1931) The biological effects of short radiation. *Quarterly Review of Biology* 6: 253–280.
Pahan, K., Ray, S., and Gachhui, R., et al. (1993) Stimulatory effect of phenylmercuric acetate and benzene on the growth of a broad spectrum mercury-resistant strain of *Bacillus pasteurii*. *Journal of Applied Bacteriology* 74: 248–252.
Pall, M.L. (1973) Sexual induction in Volvox-carteria-quantitative study. *Journal of Cellular Biology* 59: 238–241.
Pape, R. (1951) New observations and experimental findings on the effect of very small roentgen doses. *Radiologia Austriaca* 4: 35–51.
Pape, R. and Jellinke, N. (1950) Important variations in the organic findings after total and local radiation and the particular effects of small scale dosages. The spleen as a test for various radiation effects. *Radiologia Austriaca* 3: 43–62.
Pape, R., 1950. Histological findings after very small doses of irradiation. In: Proceedings of the Sixth International Congress of Radiology, London, pp. 162–163.
Peters, R.A. (1920) Variation in the resistance of protozoon organisms to toxic agents. *Journal of Physiology* 54: 260–266.
Pettis, R.J., Erickson, B.W., Forward, R.B., and Ritschoff, D. (1993) Superpotent synthetic tripeptide mimics of the mud-crab pumping pheromone. *International Journal of Peptide and Protein Research* 42: 312–319.
Pugsley, A.T., Oddie, T.H., and Eddy, C.E. (1935) The action of X-rays on certain bacteria. *Proceedings of the Royal Society of London B* 118: 276–298.
Rahn, O. (1915) Der Einfluss der Temperature und der Gifte aluf Enzymwirkung, Garung und Wachstrum. *Biochemische Zeitschrift* 72: 351.
Rahn, O. (1932) *Physiology of Bacteria*. P. Blakiston's Son and Co.,, Philadelphia, PA.
Randall, W.A., Price, S.W., and Welch, H. (1947) Demonstration of hormesis (increase in fatality rate) by penicillin. *American Journal of Public Health and the Nation's Health* 37: 421–425.
Reed, L.J. and Berkson, J. (1929) The application of the logistic function to experimental data. *Journal of Physical Chemistry* 33: 760–779.
Reichel, H. (1909) Zur theorie der desinfektion. I, II, and III. *Biochemische Zeitschrift* 22: 149–177 177–199, 201–232.
Roy, B. and Rai, U. (2004) Dual mode of catecholamine action on splenic macrophage phagocytosis in wall lizard, *Hemidactylus flaviviridis*. *General and Comparative Endocrinology* 136: 180–191.
Salle, A.J. (1939) Influence of environment upon bacteria. *Fundamental Principles of Bacteriology With Laboratory Exercises*. McGraw-Hill Book Co., Inc.,, New York.
Samson, L. and Cairns, J. (1977) New pathway for DNA-repair in *Escherichia-coli*. *Nature* 267: 281–283.
Shackell, L.F. (1925) The relation of dosage to effect. II. *Journal of Pharmacology and Experimental Therapeutics* 25: 275–288.
Shackell, L.F., Williamson, W., Deitchman, M.M., Katzman, G.M., and Kleinman, B.S. (1924/1925) The relation of dosage to effect. *Journal of Pharmacology and Experimental Therapeutics* 23 (24): 53–65.
Shalev, A.H. and Altstein, M. (2015) Pheromonotropic and melanotropic PK/PBAN receptors: Differential ligand-receptor interactions. *Peptides* 63: 81–89.
Shanmugam, G., Narasimhan, M., and Sakthivel, R., et al. (2016) A biphasic effect of TNF-α I regulation of the Keap 1/Nrf2 pathway in cardiomyocytes. *Redox Biology* 9: 77–89.
Sielken, R.L. and Stevenson, D.E. (1998) Some implications for quantitative risk assessment if hormesis exists. *Human and Experimental Toxicology* 17: 259–262.
Slikker, W., Andersen, M.E., and Bogdanffy, M.S., et al. (2004a) Dose-dependent transitions in mechanisms of toxicity. *Toxicology and Applied Pharmacology* 201: 203–225.
Slikker, W., Andersen, M.E., and Bogdanffy, M.S., et al. (2004b) Dose-dependent transitions in mechanisms of toxicity: Case studies. *Toxicology and Applied Pharmacology* 201: 226–294.
Smith, E.C. (1935) Effects of ultra-violet radiation and temperature on Fusarium II. Stimulation. *Bulletin of the Torrey Botanical Club* 62: 151–164.
Smith, J.H. (1921) The killing of Botrytis spores by phenol. *Annals of Applied Biology* 8: 27–50.
Smith, J.H. (1923) The killing of *Botrytis cinerea* by heat, with a note on the determination of temperature coefficients. *Annals of Applied Biology* 10: 335–347.
Southam, C.M. and Ehrlich, J. (1943) Effects of extracts of western red-cedar heartwood on certain wood-decaying fungi in culture. *Phytopathology* 33: 517–524.
Southam, C.M., 1941. A study of the saprogenicity, and factors influencing decay, of certain brown-rot fungi on Western red cedar heartwood test blocks. Thesis, School of Forestry, University of Idaho.
Sowa, G., Gekker, G., and Lipovsky, M.M., et al. (1997) Inhibition of swine microglial cell phagocytosis of *Cryptococcus neoformans* by femtomolar concentrations of morphine. *Biochemical Pharmacology* 53: 823–828.
Stebbing, A.R.D. (1982) Hormesis—the stimulation of growth by low-levels of inhibitors. *Science of the Total Environment* 22: 213–234.
Stebbing, A.R.D. (2009) Interpreting dose-response curves using homeodynamic data: With an improved explanation for hormesis. *Dose-Response* 7: 221–233.
Stoklasa, J. and Penkava, J. (1928) Biologie des uraniums. *Biochemische Zeitschrift* 194: 15–77.

Sukata, T., Uwagawa, S., and Ozaki, K., et al. (2002) Detailed low-dose study of 1,1-B IS(*p*-chlorophenyl)-2,2,2-trichloroethane carcinogenesis suggests the possibility of a hormetic effect. *International Journal of Cancer* 99: 112–118.

Sykes, P.J. and Day, T.K. (2007) Requirements for identification of low dose and non-linear mutagenic responses to ionizing radiation. *Dose-Response* 5: 308–314.

Szabadi, E. (1977) Model of 2 functionally antagonistic receptor populations activated by same agonist. *Journal of Theoretical Biology* 69: 101–112.

Tabakoff, B. and Kiianmaa, K. (1982) Does tolerance develop to the activating, as well as the depressant, effects of ethanol? *Pharmacology Biochemistry and Behavior* 17: 1073–1076.

Tan, H.Y., Wang, N., and Li, S., et al. (2016) The reactive oxygen species in macrophage polarization: Reflecting its dual role in progression and treatment of human diseases. *Oxidative Medicine and Cellular Longevity* 2016: 1–16. https://doi.org/10.1155/2016/2795090.

Thol, W., 1885. Ueber den Einfluss organischer, nichte aromatischer Sauren auf Gahrung und Faulnis. Inaugural Dissertation, Greifswald, Germany.

Timoféeff-Ressovsky, N.W., Zimmer, K.G., and Delbruck, M. (1935) Uber die natur der genmutation und der genstruktur. Nachrichten von der Gesellschaft der Wissenschaften zu Gottingen: Mathematische-Physikalische Klass, Fachgruppe VI. *Biologie* 1: 189–245 [English translation: On the nature of gene mutation and gene structure. Reprinted in Sloan PR and Fogel B (eds.) (2011) Creating a physical biology. The three-man paper and early molecular biology. Chicago: The University Press of Chicago.

Toimela, T. and Tähti, H. (2004) Mitochondrial viability and apoptosis induced by aluminum, mercuric mercury, and methylmercury in cell lines of neural origin. *Archives of Toxicology* 78: 565–574.

Trevan, J.W. (1927) The error of determination of toxicity. *Proceedings of the Royal Society of London B* 101: 483–514.

Ugazio, G., Koch, R.R., and Recknagel, R.O. (1972) Mechanism of protection against carbon tetrachloride by prior carbon tetrachloride administration. *Experimental and Molecular Pathology* 16: 281–285.

Ugazio, G., Koch, R.R., and Recknagel, R.O. (1973) Reversibility of liver damage in rats rendered resistant to carbon tetrachloride by prior carbon tetrachloride administration: Bearing on the lipoperoxidation hypothesis. *Experimental and Molecular Pathology* 18: 281–289.

Ullah, F., Gul, H., and Tariq, K., et al. (2020) Thiamethoxam induces transgenerational hormesis effects and alteration of genes expression in *Aphis gossypii*. *Pesticide Biochemistry and Physiology* 165: 104557.

US National Academy of Sciences (US NAS), 1977. Drinking Water and Health. Safe Drinking Water Committee, Washington DC.

Varlinskaya, E.I., Spear, L.P., and Spear, N. (2001) Acute effects of ethanol on behavior of adolescent rats: role of social context. *Alcoholism, Clinical and Experimental Research* 25: 377–385.

Wakabayashi, N., Slocum, S., Skoko, J., Shin, S., and Kensler, T. (2010) When NRF2 talks, whose listening? *Antioxidative Redox Signaling* 13: 1649–1663. https://doi.org/10.1089/ars.2010.3216.

Warren, S. (1945) The histopathology of radiation lesions. *Physiological Reviews* 25: 225–238.

Wels, P. (1933) The life time work of Hugo Schulz. *Naunyn-Schmiedebergs Archiv fur Experimentelle Pathologie und Pharmacologie* 170: 744–757.

Wyckoff, R.W.G. (1930) The killing of certain bacteria by x-rays. *Journal of Experimental Medicine* 52: 435–447.

Yeang, H.X.A., Hamdam, J.M., and Al-Huseini, L.M.A., et al. (2012) Loss of transcription factor nuclear factor-erythroid 2 (NF-E2) p45-related factor-2 (Nrf2) leads to dysregulation of immune functions, redox homeostasis, and intracellular signaling in dendritic cells. *The Journal of Biological Chemistry* 287: 10556–10564.

Yule, G.U. (1910) On the distribution of deaths with age when the causes of death act cumulatively, and similar frequency distributions. *Journal of the Royal Statistical Society* 73: 26–38.

Zheng, G., Ren, H., and Li, H., et al. (2019) *Lycium barbarium* polysaccharide reduces hyperoxic acute lung injury in mice through Nrf2 pathway. *Biomedical Pharmacology* 111: 733–739.

Zhu, X.-W., Liu, S.-S., Qin, L.-T., Chen, F., and Liu, H.-L. (2013) Modeling non-monotonic dose-response relationships: Model evaluation and hormetic quantities exploration. *Ecotoxicology and Environmental Safety* 89: 130–136.