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Hormesis: principles and applications

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Hormesis has emerged as a central concept in biological and biomedical sciences with significant implications for clinical medicine and environmental risk assessment. This paper assesses the historical foundations of the dose–response including the threshold, linear and hormetic models, the occurrence and frequency of the hormetic dose response in the pharmacological and toxicological literature, its quantitative and temporal features, and underlying mechanistic bases. Based upon this integrative foundation the application of hormesis to the process of risk assessment for non-carcinogens and carcinogens is explored. *Homeopathy* (2015) **104**, 69–82.

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Introduction

The most fundamental concept in toxicology is the dose response relationship.[1] Hazard and risk assessment is based on an understanding as well as assumptions of the dose response relationship. Mechanistic understanding within toxicology is often directed to providing explanations of why the dose response displays specific dose-related transition characteristics.[2,3] For nearly a century it has been widely accepted that the dose–response relationship follows a sigmoidal or S-shaped pattern of response with the tails of the lower and upper ends of the distribution asymptotically approaching zero and 100%, respectively.[4] The approaching of a zero response in the low dose domain suggested both the theoretical and practical existence of a threshold dose below which there would be no treatment related effects. Despite its long term acceptance in toxicology and pharmacology, developments over the past two decades suggest the possibility that alternative dose response models may better account for observed dose responses in the low dose zone. This article will assess the evolution of the dose–response concept, including the origin of the threshold dose response model, the subsequent proposal of the linear dose response model to assess mutagenic and carcinogenic responses and the recent resurgence of the biphasic hormetic dose response. Particular emphasis will be directed toward how the hormetic dose response may affect the process of risk assessment for both non-carcinogens and carcinogens. The paper will also suggest a means by which the hormetic and LNT models may be functionally integrated to assess low dose cancer risks.

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Historical foundations of the dose-response relationship

Amongst the earliest and most substantial support for the threshold dose response model was reported by Shakell.[5–7] The conclusions of Shakell and colleagues were expanded by Alfred J. Clark, the British pharmacologist, whose influential textbooks were to affect his contemporaries and subsequent generations of pharmacologists and toxicologists.[4,8,9] The concept of threshold became further entrenched in toxicological thought when the highly influential dose response model, probit analysis, that was created in the 1930s, independently by Bliss[10,11] and Gaddum,[12] incorporated the concept of maximum likelihood via the influence of the renowned biostatistician Ronald Fisher in an appendix to a paper by Bliss.[10]

This process resulted in the probit model incorporating statistical assumptions that below control responses were components of biological variation and not to be assessed as real treatment effects, constraining the response in the low dose zone to approach zero. This meant that the only "real" biological responses in the low dose zone were those approaching zero while below control responses were manifestations of variability and/or error in the experimental systems. By the early 1940s researchers from the newly formed U.S. National Cancer Institute (NCI) (i.e., created in 1938) were applying the Fisher-based imposed statistical constraints of the probit model to estimate responses to carcinogenic hydrocarbons even though the data indicated tumor responses below control values in the low dose zone.[13]

These concepts came to dominant the intellectual foundations of modern toxicology, becoming institutionalized in subsequent major textbooks in pharmacology such as Goodman and Gilman and in toxicology such as Casarett and Doull. Their influence permeated the recommendations of major advisory organizations such as the U.S. National Academy of Sciences as especially seen in publications of the Safe Drinking Water Committee[14] and in the actions of major regulatory agencies.

The consequences of this consolidated dose–response belief system were profound. Most importantly, it provided the theoretical foundations to establish modern toxicology as a high dose, few doses discipline. It established the goals of hazard assessment testing to include the estimation of the lowest observed adverse effect level (LOAEL) (i.e., lowest dose that is statistically significantly different than the control group) and the no observed adverse effect level (NOAEL), that is, the highest dose that does not differ significantly from the control group.

A system of uncertainty factors (UF) was built within this dose–response framework by the mid 1950s based on the recommendations of the U.S. FDA toxicologist, Arnold Lehman[15–17] after whom the most prestigious annual award of the U.S. Society of Toxicology is named; one could readily derive an acceptable level of exposure once having obtained both NOAEL/LOAEL estimates or, in fact, either one. This hazard assessment framework could be conducted in a cost effective manner since the size of experiments could be modest based on the assumed threshold nature of the dose response in the low dose zone, thereby limiting the size (e.g., number of doses, sample size) of experimental studies and not including repeat measures.

Linearity at low dose challenges the threshold model

The threshold dose response model has been the dominant dose response model in toxicology for about 80 years. However, its incorporation into government mandated frameworks for testing chemical products that will enter society and how subsequent risks may be assessed has not been without controversy. The most significant and successful challenge to the threshold model involved how carcinogen risks are estimated. As early as the 1950s highly influential organizations (i.e., NCRPM[18]; NAS[19]) assessing the biological effects of radiation on human health rejected the long standing assumption of a threshold response (i.e., referred to as the tolerance dose),[20] based upon findings that radiation induced mutations in a linear manner and that mutations were a necessary mechanism or stage in the process of carcinogenesis.[21,22]

These concepts eroded the underlying belief in the threshold model leading to a probabilistic framework for assessing low-dose cancer risks from radiation. This approach eliminated the concept of a "safe" dose of radiation as far as mutagenicity and carcinogenicity were concerned and replaced it with the concept of "acceptable" risk. Safety, that is, the absence of risk, could no longer be guaranteed unless the exposure was zero. With this new framework for evaluation, acceptable risk became a judgmental perspective based on personal, political, and cultural values and complex societal trade-offs.

The concepts of a linear dose–response at low doses and acceptable risk, as developed in the field of radiation biology, also permeated the domains of chemical toxicology and risk assessment. By the mid 1970s the Safe Drinking Water Committee of the U.S. National Academy of Sciences published an influential book entitled *Drinking Water and Health* in which it applied the linear dose response concept to chemically-induced carcinogenesis. Systems of evaluation were created using a variety of competing biostatistical models that assumed linearity at low dose. This application of linearity at low dose modeling was nothing short of revolutionary for toxicology and risk assessment within the U.S. and the rest of the industrial world. Risk assessments and regulations for numerous chemical carcinogens came forth which reflected this new probabilistic approach for cancer risk assessment.

These developments led to the coining of phrases in the 1980s such as "how clean is clean", as related to the extent to which environmental contamination would have to be remediated to achieve acceptable cancer risk levels such as 10^{-5}, 10^{-6} or others. Other major impacts of linearity at low dose modeling in non-industrial countries affected decisions on the use of carcinogenic pesticides for the eradication of insect borne diseases such as malaria, a disease still annually affecting millions.[23] Risk assessments based on linearity at low doses created a framework for the elimination of the high dose rodent carcinogen DDT, even when proposed for use in very restrictive ways, though it has been strongly argued as the most cost-effective means to prevent life threatening malaria in numerous situations.

Based on this change in the concept of dose–response for radiation and chemical carcinogenesis, cancer risk assessment became the driver for environmental cleanup costs and many governmental policy decisions. Permissible exposures for carcinogens were placed within an evolving regulatory framework in which the goal of exposure standards for carcinogens was becoming zero. For example, the drinking water standards of the US EPA have as their goal for chemical carcinogens a zero exposure limit[24] under the belief that the nature of the dose response is linear at low dose and therefore even a single molecule may have a risk that can be estimated, though not measurable.

The linearity at low dose challenge to the threshold dose response model has been enduring and profound. Despite its controversial nature and significant economic implications, it has been successfully incorporated within a broad range of regulatory agencies and advisory committees. This continues to be evidenced as seen in the 2006 report of the BEIR VII Committee of the National Academy of Sciences/National Research Council, which reaffirmed its support for the linearity at low dose hypothesis for ionizing radiation.[25]

A key scientific component of this challenge to the threshold model should require that predictions based on low dose linearity be evaluated and validated. In practice, this is never the case. The chronic bioassay employs up to three to four doses, each a fraction (1/8 to 1/2) of the maximum tolerable dose (MTD), that is, the highest dose that is not expected to cause toxicity over a normal experimental lifespan. Thus, the typical rodent bioassay does not address the issue of ambient or normal exposures, which are far less (i.e., possibly up to 4−6 orders of magnitude lower than experimental doses). The chronic bioassay is simply a high dose hazard assessment protocol; its relevance to the human condition is based on tenuous assumptions concerning the nature of the dose response in the low dose zone.

This lack of dose realism in the underlying foundation of the chronic bioassay has not gone unnoticed by governmental, industrial or academic toxicologists. The high dose protocol of the rodent bioassay may cause tissue-specific damage at high doses with the repairing of such damage initiating potentially significant tumor promotion processes.[26] This type of tumor promoting reparative response may affect low dose cancer modeling results in overestimating risks at low doses based on the linear at low dose methodology.

Linearity at low dose modeling has difficulty in developing adequate and cost-effective means for testing or validating its predictions. The achievement of this goal has been a profound failure. Testing at doses estimated to fall into the range of acceptable risks to humans would typically require vast numbers of mice and/or rats, being costly and time consuming. Since resources are limited, such large experimental studies on one agent would impact the availability of testing that could be performed with other agents.

Nonetheless, the U.S. government accepted the challenge in the late 1970s to resolve the issue of what is the nature of the dose response in the low dose zone for genotoxic chemical carcinogens. The strategy involved testing the rodent carcinogen acetylaminoflourene (AAF) in the largest rodent bioassay (24,000 mice).[27] This evaluation became known as the ED01 study because the extent to which risk could be confidently measured was only to one in one hundred rather than one in a million, the intended hope of the regulatory agencies. Since a 24,000 mouse study could only offer limited predictive values for low risks, it soon became evident that low dose linearity predictions would remain simply that, predictions that could not be validated. From a public policy perspective, this is problematic since governmental risk assessment programs and numerous activities throughout society are based on a high dose testing methodology that could never be validated in a practical sense under most conditions.

The validation of low dose predictions is also problematic for human epidemiological investigations. It is generally accepted that confident estimates of disease incidence in epidemiology cannot be made until the estimated risks start to exceed a factor of two to three. Yet, the acceptable risk concept that EPA and FDA have adopted have typically related to risks in the vicinity of 0.000001 (one in a million) and not the 2- or 3-fold zone that epidemiology can only usually reliably provide.[28] Thus, validation of carcinogen risk by epidemiological methodology is simply not possible at least as far as low risks.

The net result of these two converging and complementary methodologies does not permit regulatory agencies to validate risks in the low risk zone that society finds acceptable and necessary. Government estimations of risk in the low dose zone are based on assumptions of the nature of the dose response, decisions not derived from data but by belief systems (i.e., Precautionary Principle).

Hormesis challenges threshold and linearity models

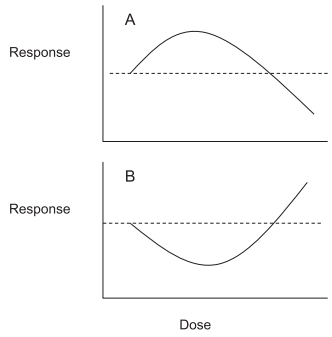
Over the past several decades regulatory agencies such as the EPA and FDA have developed a dual track for chemical risk assessment. One track deals with the assessment of non-carcinogens which is dealt with by assuming such agents act via a threshold mechanism; the second deals with the assessment of carcinogens which assumes that such agents act via a non-threshold mechanism.

Definition of hormesis

Despite the codifying of these two approaches for assessing chemicals and radiation, a new challenge has emerged which claims that neither the threshold nor the linearity at low dose response models are the most basic and common models of dose response relationships for non-carcinogenic and carcinogenic endpoints. This challenge claims that the most fundamental dose response model in the biological sciences, including toxicology, is the hormetic dose−response relationship. **The hormetic dose−response may be defined as a low dose stimulation and a high dose inhibition, that is, a biphasic dose−response relationship. The form of this relationship may be either an inverted U-shaped or a J-shaped dose−response** (Figure 1).[29]

Whether this dose−response relationship is an inverted U- or J-shaped depends on the particular endpoint being measured and the manner in which it is presented. For example, if longevity, learning, growth or fecundity were assessed and graphed these would typically appear as following an inverted U-shaped dose response. However, if the endpoints measured were disease frequencies such as tumor, birth defects or heart disease then the dose-responses would typically be presented as J- or U-shaped. Both of these general types of responses are examples of hormetic dose−response relationships.

Another feature of the hormetic dose response is that it contains two thresholds. One is the traditional toxic threshold seen with the threshold model or the zero equivalent point (ZEP), this is the dose where the response crosses the control group response and becomes toxic/inhibitory. The second threshold occurs at a lower dose than the traditional toxic threshold, that is, when the stimulatory response decreases and eventually regresses to



(A) The most common form of the hormetic dose-response curve depicting low-dose stimulatory and high-dose inhibitory responses, the β- or inverted U-shaped curve.

(B) The hormetic dose-response curve depicting low-dose reduction and high-dose enhancement of adverse effects, the J- or U-shaped curve.

Figure 1 Hormetic dose response relationships.[76]

become indistinguishable from the control value (Figure 1). This represents an important feature that has potential implications for the risk assessment process as well in clinical medicine.

Hormetic model

Overcompensation stimulation hormesis

In the case of the hormetic model there may be a critical temporal component, requiring a dose-time-response evaluation. This is based on observations that the hormesis stimulatory response in the low dose zone (i.e. below the traditional toxic threshold) can result from an overcompensation following an initial disruption in homeostasis (Figure 2). With respect to the time component of overcompensation hormesis, the dose–response is a series of time-based snap shots. Immediately after exposure to the toxic substance, there is often induced toxicity.[30] Such toxicity could exhibit an initial dose–response relationship that reflects either a threshold-like or linearity at low dose response.

However, at subsequent time points compensatory responses often become evident and the dose–response begins to display a rebound-like process which ultimately leads to the hormetic biphasic dose–response. Repair/compensatory processes are initiated following the induction of damage across the entire dose response. At doses below the traditional threshold, the compensatory response is sufficient to repair fully the damage. Not only is the induced damage often eliminated but background damage may also diminish thereby resulting in less damage than to start with.

The conclusion that the hormetic stimulation in the below threshold zone is the result of an overcompensation or a rebound response has implications affecting the quantitative features of the hormetic stimulatory response. During repair, biological processes lead to the re-establishment of homeostasis, having both time and resource implications. In the case of biological resources the affected system might be expected to allocate enough resources to ensure complete recovery in a timely and efficient fashion via complex cybernetic processes.

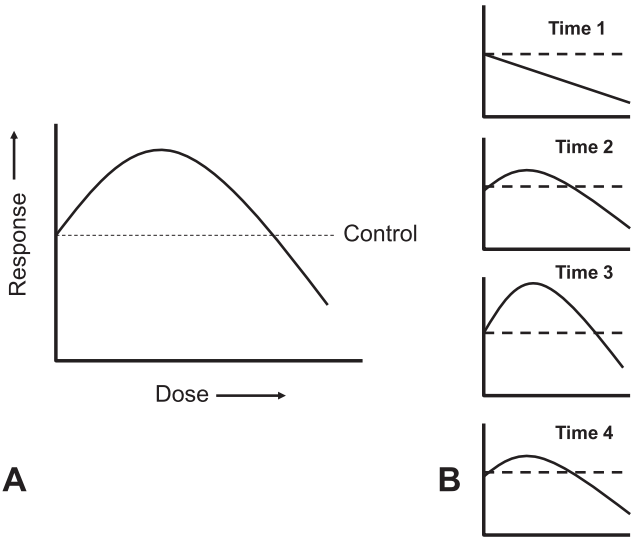


Figure 2 Overcompensation stimulation (hormesis) within a dose-time-response relationship. Response (R) on the vertical axis, dose (D) on the horizontal axis.[36] This mechanism indicates that initially at time 1, there is a dose dependent decrease consistent with a toxic response after the initial exposure to the toxic agent. Then, at time 2 there is a slight overcompensation within the low dose range, followed by a maximum increase over the controls at time 3. If we were to follow this dose response over more time periods, it may return to control values.

Large numbers of examples of hormetic dose response relationships reveal that the magnitude of overcompensation is modest resulting in stimulatory responses in the magnitude of "percentages" rather than "fold" increases.[31–33] These observations indicate a high degree of efficiency, accounting for the limited "overshoot" phenomenon (i.e., low dose stimulation). In the majority of cases the maximum stimulatory responses are only about 30–60% greater than the control response (Figure 3). In only a minority of cases in which the compensatory responses occur is there an increase over controls exceeding two fold. This may reflect an organism that is not as efficient as those which display the more modest response. One may speculate that this larger response may be a marker for aging processes or concurrent disease activities.

The overcompensation response, besides representing a modest over-allocation of resources for repairing damage, may be integrated into other processes leading to a reduction in background damage, or to enhance resistance to subsequent and more harmful exposures as seen with preconditioning.[34] This process accounts for the J-shaped response seen in the reduction of disease incidence to certain carcinogens[35,36] and the process of adaptive response to radiation, chemical toxicities with preconditioning and various clinical pathologies (e.g., heart attack, stroke). From an evolutionary perspective this process would confer an advantage to an organism that first encounters a dilute concentration of a toxic substance prior to encountering a more concentrated and threatening form of the toxic substance. The induced adaptive/preconditioning response also displays the inverted U-shaped dose response.[34]

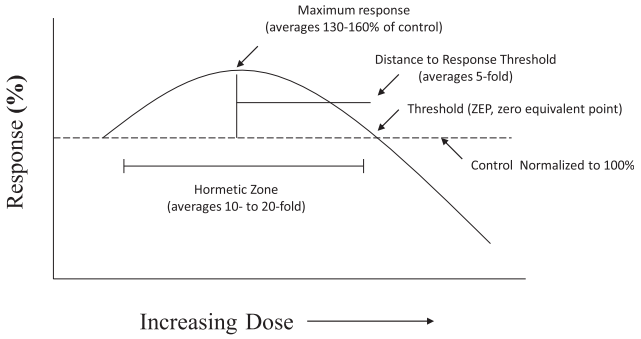


Figure 3 Dose-response curve depicting the quantitative features of hormesis and its application to the concept of enhanced biological performance.[77]

The overcompensation feature of the hormetic dose response has often been missed or under-appreciated in traditional toxicological research. The overcompensation hormetic dose response places significant demands on the investigator with respect to the quality of the study design. There is a need for sufficient doses to define the upper end of the toxicity curve as well as the below threshold component of the dose response. In addition, it is necessary to have an adequate number of time points over which to measure the toxicity and repair processes that combine to define the dose-time-response relationship. However, traditional approaches to hazard assessment as imposed by federal regulatory agencies in the assessment of chemical and pharmaceutical agents have been guided by belief in the traditional threshold model, a perspective challenged by the hormetic dose response model, using study designs that are unable to adequately assess possible hormetic dose-responses.

Direct stimulation hormesis

While a large number of hormetic dose responses result from an overcompensation to a disruption in homeostasis, the strong majority employ multiple doses but with only one time point.[31–33] In such cases, the biphasic dose response appears as a direct stimulation. There are also many examples of low dose stimulatory responses occurring quickly after exposure, thereby suggesting that direct stimulation of hormetic responses are common, especially in the pharmacology literature. Whether the hormetic dose response occurs either as a result of an overcompensation response or a direct stimulation, the quantitative features of the dose response are similar.

Width of the hormetic stimulatory response

Another feature of the hormetic dose response relationship is the width of the stimulatory response. In contrast to the generally consistent limited magnitude of stimulation in the hormetic zone, the width of the stimulatory response can be quite variable. The majority (i.e., ~70%) of observed stimulatory ranges are within 1/100th of the traditional threshold. Approximately 5% of the cases display stimulatory widths that exceed 1/1000th of the threshold (i.e., ZEP) value (Figure 4). The underlying causes of variability in the width of the hormetic dose response are generally unexplored. Simulations of differential population susceptibilities have indicated that the width of the stimulatory response can be associated with the heterogeneity of the population.[37] This suggests that most hormetic stimulatory widths are modest because the experimental models are reasonably genetically homogeneous. The hormetic stimulatory range might be expected to be larger in outbred rather than in inbred animal model strains.

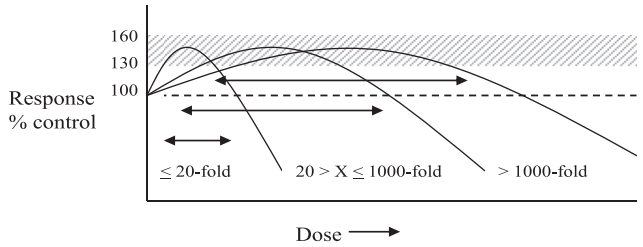


Figure 4 Stylized dose–response curves reflecting the relative distribution of stimulatory dose ranges. Note: The maximum stimulatory response is usually 130–160% of the control value.[35]

There are some examples of wide stimulatory ranges in animal models exposed to agents, such as endocrine modifying substances.[31,33] One possible explanation for large stimulatory ranges may relate to the heterogeneity of the population with respect to the developmental period when the organism is susceptible to the induced biological effect. For example, if the window of higher activity is only a few hours or even minutes, it is possible that a wide inter-individual variability could develop, resulting in a broad stimulatory range.

Historical foundations of hormesis

Origins and terminology

Credit for creating the experimental basis of the hormesis concept has typically gone to Hugo Schulz, a traditionally trained physician/pharmacologist at the University of Greifswald in Northern Germany, for his research with yeast in the 1880s[38–40](translation of Schulz 1923 autobiography). Schulz reported the effects of numerous disinfectants on yeast metabolism as measured by the release of carbon dioxide. At low concentrations there was a highly reproducible stimulation of metabolism while being inhibited at the higher concentrations (see Branham[41] who replicated Schulz's original findings).

The term hormesis was not applied to dose–response relationships with a low dose stimulation and a high dose inhibition until 1943 when graduate student Chester Southam and Professor John Ehrlich, then at the University of Idaho, used this term (i.e., meaning to excite) to describe the effects of extracts of the red cedar tree on the growth of multiple fungal species.[42] The earliest tracing of the term occurred two years earlier in Southam's undergraduate thesis.[43]

Linking hormesis with homeopathy

Despite credit for discovering the concept, although not the name, of hormesis, Schulz also created considerable

difficulties for its acceptance by immediately linking it to the medical practice of homeopathy. Schulz believed that he had uncovered the underlying scientific foundation for homeopathy. However, as straight-forward as his reproducible scientific findings seemed to be, the application of this dose–response concept to the field of homeopathy was anything but uncontroversial because of the intense and prolonged rivalry between what is now called "traditional" western medicine and homeopathy. By "positioning" the biphasic dose–response phenomenon in the "political" camp of homeopathy, Schulz and this concept became politicized and the object of ridicule and rejection by intellectual leaders in pharmacology, a mainstay of traditional medicine. This may be seen in the writings of Clark[4,8,9] which questioned the reproducibility of many biphasic dose responses. Even when such findings were reproducible, Clark challenged their biological significance, further trivializing this dose–response phenomenon.

By all appearances the efforts of Clark to minimize the influence of Schulz's leadership on the nature of the dose–response were highly successful. This was achieved by unbalanced representations of homeopathy which Clark defined only within the context of its most extreme elements of dose response understandings (i.e., responses to doses below Avogadro's number-10^{-23} M), a concept which had been rejected by the majority of homeopathy practitioners well before Clark's professional career.[44] By linking Schulz's work to the extremist elements of homeopathy and associating homeopathy with quackery (Clark, 1926), the scientific concepts put forward by Schulz were seriously compromised. Of considerable importance was that Clark was a very highly regarded professional within the British pharmacological community, being one of its founding members, author of several highly regarded textbooks and head professor of pharmacology at a prestigious institution in the United Kingdom, the University of Edinburgh.

It is important to appreciate that toxicology had its origins as a natural outgrowth of pharmacology thereby predisposing it to reject the findings of Schulz. The work of Clark cast a long and dominating shadow over the fields of pharmacology and toxicology during the 1920s to the 1950s, a period of concept consolidation within toxicology. The first generation of U.S. and European toxicologists were educated and trained as pharmacologists, only to latter acquire the application to toxicology. Educated within the framework of traditional medical education, they saw homeopathy as a fraudulent medical practice, lacking scientific foundation and associated the work of Schulz (i.e., his biphasic dose response) with the extremist elements of that discredited practice.

During the mid 20th century the concept of dose response became better understood, modeled, and institutionalized within academic settings and governmental agencies. Frameworks were developed for the hazard assessment of chemicals and drugs and for what today is called risk assessment. The striking absence of the concept of hormesis from major textbooks of pharmacology and toxicology throughout the entire 20th century is a testimony to the success of Clark and his colleagues and the unfortunate lack of scrutiny of the dose response literature, especially in the low dose zone by subsequent generations of pharmacologists and toxicologists. The marginalization of the hormesis concept during the latter half of the 20th century was evident not only by its absence from major texts but also in its omission from major professional society meetings, lack of funding by federal agencies, and rejection by regulatory agencies.

Despite significant setbacks and obstacles to its understanding and acceptance, the concept of hormesis did not disappear. There was a continuing publication of articles in the scientific literature over the past century providing documentation and support for the original concept of Schulz.[45,46] These early developments were principally seen in the fields of plant biology, microbiology and entomology.[47–51] So common were the observations of a low dose stimulation and high dose inhibition that it became a standard laboratory bioassay in introductory microbiology laboratory courses.[52,53]

Evolution of the hormesis concept

Even though the biphasic dose response of Schulz was often observed by researchers and published in scientific journals, accepted terminology to describe this concept was never achieved. That is, the phenomenon of Schulz became known as the Arndt-Schulz Law, after Schulz and his homeopathic physician colleague, Rudolf Arndt. It also became known by a rival term, Hueppe's Rule, after Ferdenande Hueppe, a well known bacteriologist, with training and association with the Nobel laureate Robert Koch.[54]

These initial descriptors have been gradually replaced by the term hormesis as noted earlier. However, this term was not adopted very quickly being little cited in the intervening years. The modern revival of this term occurred, in part, as a result of the efforts of Luckey who wrote two books on ionizing radiation and hormesis in 1981 and 1992. Prior to the 1990s the term hormesis was rarely cited, even by those who published supportive findings. However, in 2013 the terms hormesis or hormetic were cited nearly 6000 times in the Web of Knowledge/Science database, whereas throughout the 1980's it was cited in this database only about 10–15 times per year.

While the term hormesis has become more widely used over the past decade, there has been no obvious consensus on what term should be used to describe biphasic dose–response relationships. In fact, there are many terms that have been used to describe this general type of dose–response relationship, including hormesis, mitohormesis, biphasic, bell-shaped, non-monotonic, ambiguous effect, bitonic, bimodal, dual effects, stimulatory-inhibitory, U-shaped, J-shaped, inverted U-shaped, Yerkes-Dodson Law, as well as the Arndt-Schulz Law and Hueppe's Rule, amongst others. This use of many terms, which are often scientific discipline specific, has created communication and understanding challenges.

The lack of a common terminology for the same concept of the biphasic dose response underscores the fact that most researchers are not aware that this concept is a very general one and that specific biphasic dose response relationships may be manifestations of a similar biological principle.

Hormesis should be value neutral

Since hormesis is a dose–response phenomenon, it should be value neutral. Many publications have referred to hormesis as a beneficial effect at low doses. The perspective offered here is that hormesis is a scientific concept which should be decoupled from whether the phenomenon is interpreted as conferring benefit or harm, since both are possible depending on the situation.[29] For example, if a chemical enhanced the proliferation of a harmful bacteria within a human, it may be beneficial for the bacteria but harmful to the human.

Take the case of the theoretical hormetic increase in human longevity by 20%. While on the individual level this would likely be seen as a beneficial effect, however, such an increase may be extremely difficult for society and currently constructed governmental programs, to deal with effectively, possibly creating more harm than benefit. The determination of whether the low dose stimulatory effect of the hormetic dose response confers possible benefit or harm is important to resolve but should not be a part of the definition of hormesis itself.

Regulatory agencies and low dose beneficial responses

Over the past two decades there has been a major refocusing on the concept of hormesis. The principal motivation for this interest has been, at least in part, a response to extremely conservative risk assessment practices by regulatory agencies with respect to carcinogen regulation. As discussed above, regulatory agencies have adopted a linearity at low dose policy for assessment of carcinogenic risks. Low levels of risks such as one cancer per million people per 70-year period have become commonplace implementation standards worldwide. Such practices often result in expensive technologies and/or cleanup activities. In fact, carcinogenic risk assessment practices are the principal drivers in the risk assessment process and the cause of the vast resources required to comply with regulatory decisions.

The regulatory community countered the linearity at low dose perspective, arguing that it was more likely that carcinogen responses at low doses behaved in a threshold manner. However, this strategy of opposition to carcinogen policy and practices has been generally a failure. Regulatory agencies have invariably rejected a conclusion of threshold for carcinogen activity since the amount of available data with almost any individual experiment is inadequate to reliably distinguish the threshold from the linearity at low dose model. When the two models cannot be confidently distinguished regulatory agencies have been guided by a protectionist philosophy, erring on the side of their perception of safety-given the two options.

In the mid 1980s an alternative strategy that evoked the concept of hormesis was proposed. Since it was not practically possible to replace linearity at low doses with the threshold model, some thought it may be possible to achieve this goal with the hormetic model. It was reasoned that it would be more likely to confidently distinguish the hormetic response from linearity at low doses since the two responses would differ more than linearity vs threshold. Secondly, if hormesis were a reproducible phenomenon, a threshold for carcinogenesis could be demonstrated. While hormesis was to be the vehicle for switching from a linearity to a threshold model, the focus was less on achieving the optimal decrease in tumor response at the nadir of the hormetic dose response than in eliminating the linearity concept from regulation.

Regulatory agencies such as the U.S. EPA countered this strategy by affirming that the goal of a risk assessment is to reduce the probability of harm; they also indicate that this process should not take into consideration the fact and/or even the possibility of beneficial effects from toxicant exposure. Thus, the EPA would not incorporate hormetic effects at the low end of the dose response even if biologically significant and reproducible.[55,56] Such a course of action raises the question of whether the goal of risk assessment should be to reduce risk or maximize health? In the case of EPA a decision has been made to ignore potential health benefits of low dose exposures. The implications of such a decision are important since it contradicts protectionist philosophies as embodied in the Precautionary Principle. By ignoring the low end of a dose response relationship which reflects an hormetic response resulting in a potential benefit, EPA policy increases risks at low dose.

Threshold model predictions fail in the below threshold zone

The threshold model assumes that there is a toxic threshold, that there are biological effects at doses greater than the threshold (i.e., effects that characterize the S-shaped portion of the dose response relationship), and that there are no treatment-related effects at doses/concentrations less than the estimated threshold dose. The hormetic dose response is similar to the threshold model starting with the traditional toxic threshold dose and at subsequently higher doses. The difference between these two dose response models occurs at doses below the threshold. While the hormetic dose response model may be considered a specific type of threshold model, it also proposes that there are treatment effects below the threshold.

Three large studies have assessed the assumption of the threshold dose response model that responses below the threshold should display random variation on either side of the control value. These investigations have revealed that responses below the threshold display a non-random distribution that is consistent with the hormetic dose

response model. In the first study Calabrese and Baldwin[57,58] established *a priori* entry and evaluative criteria in order to estimate the frequency of hormetic dose responses. Of the nearly 21,000 studies evaluated from three peer-reviewed toxicology journals from their inception to the present, only two percent satisfied the entry criteria. Approximately 40% of those dose responses (~800) satisfying the entry criteria also satisfied the evaluative criteria. Amongst the reasons for the low proportion of the dose responses satisfying the entry criteria was that most lacked the necessary number of doses to evaluate responses at less than threshold doses.

Unless the dose–response had established a LOAEL, NOAEL and at least two doses below the NOAEL it would not satisfy the minimum criteria for evaluation. In a subsequent evaluation of these data Calabrese and Baldwin[58] determined the proportion of responses to doses below the threshold that were greater or less than control responses. The threshold model predicts that there should be a random distribution of responses below the NOAEL, that is, the ratio of above to equal/below control responses should be very close to 1:1 if the threshold model is correct. However, the ratio of the nearly 1800 doses below the NOAEL response from the ~800 dose responses was approximately 2.5 to 1 rather than the threshold model prediction of 1 to 1. These findings indicated that the threshold model failed to predict the response pattern in the below threshold range of the dose response. However, this response pattern was consistent with the hormesis model.

The second direct testing of the threshold versus the hormetic dose response model involved an assessment of an anti-tumor agent screening database of the U.S. National Cancer Institute (NCI).[59] This database contains the results of bioassays which tested nearly 2200 chemical agents on thirteen different strains of yeast measuring proliferation. Each chemical was tested twice in an independent fashion to create an original concentration response and its replication. Each dose response consisted of a set of averaged control groups and five concentrations. The total number of concentration response relationships comprising this database is approximately 57,000.

Using a comparable methodology to the earlier study, about 50–60% satisfied the *a priori* entry criteria depending on the yeast strain and about 70% of these displayed evidence of hormesis. The ratio of above to equal/below control responses in the thirteen yeast strains was nearly 2.5 to 1, a value inconsistent with threshold model predictions but in accordance with hormetic dose response model predictions. The third study directly testing the below threshold response predictions of the threshold dose response model assessed nearly 2000 antibiotics in *Escherichia coli*. Using similar protocols to that described for the yeast data, the below threshold responses were again remarkably inconsistent with the threshold model while highly supportive of the hormetic perspective.[60]

The above studies are significant because they are the only large scale studies in which the validity of threshold predictions have been tested. Furthermore, the data that were evaluated had to satisfy *a priori* entry criteria, thereby yielding an estimate of the frequency of threshold or hormetic responses in the toxicological literature. In each case the threshold dose response model performed quite poorly. In light of such evidence based on head-to-head evaluations one must question the basis of beliefs in the validity of the threshold model.

There are thousands of other published findings in the peer-reviewed literature that display non-random below threshold responses that violate the predictions of the threshold model. Many of these studies have been analyzed and integratively reviewed.[33,44,58,61,62] Given how important the threshold model is in guiding the fields of pharmacology, toxicology and risk assessment, how did such advanced fields make such a fundamental error on the central pillar of their respective disciplines?

Evidence supporting hormesis

Criteria to establish hormesis: study design, statistical power, replication and mechanism

In order to assess the hormetic hypothesis it is necessary to know when an hormetic dose response occurs. However, there are no criteria that unequivocally determine that hormesis has been induced. An important challenge for demonstrating hormesis is that the modest low dose stimulation may result from random variation rather than a true treatment effect. Decisions on whether hormesis occurs, requires the biological model to have an hormetic-like biphasic dose response along with a robust study design, adequate statistical power, and reliable replication.[31–33]

In many cases the degree of proof has been extended to the level of mechanism in which the administration of receptor antagonists and cell signaling pathway inhibitors have been employed to deconstruct and to reconstruct the hormetic dose response. Calabrese[63] has recently published specific mechanisms for 400 different hormetic dose responses. This documentation of a vast range of mechanisms for hormetic dose responses addresses one of the long-standing criticisms of the hormesis concept.

Hormesis database

The evidence that supports hormesis can be difficult to obtain since there is no common terminology for this concept along with the above noted lack of definitive criteria which unequivocally establishes its existence. In addition, there are large numbers of cases in which investigators have not recognized possible hormetic effects within their data nor mentioned it in their results and discussion. As a result of this unique set of circumstances, an hormesis database was created to assemble likely hormetic dose responses in a relational retrieval system. A detailed description of this database is given by Calabrese and Blain.[31–33]

The database uses *a priori* evaluative criteria to assess whether dose responses display likely evidence of hormesis. Numerical criteria were created based on study design, statistical significance, magnitude of stimulatory response

in the below threshold domain of the dose response, presence of a NOAEL and reproducibility of the findings. To date, there have been approximately 9000 dose responses that have satisfied the entry criteria and comprise the database. In general, the information obtained from the database is substantial and provides a firm basis for a number of general conclusions. Hormetic responses are numerous within the toxicological literature and generalizable, being widespread across a broad range of biological models such as plants, microbes, invertebrates and vertebrates, encompassing a large number and range of endpoints, with examples found in hundreds of chemicals, across a broad range of chemical classes. Hormetic effects have no obvious restriction and are independent of biological model, endpoint, mechanism, and chemical class as well as physical agent.

The database provides numerous examples of hormesis that permit reliable conclusions to be made concerning the quantitative features of the stimulatory response. That is, the database has been used to establish the magnitude and width of the stimulatory response as well as the relationship of the maximum stimulation to the toxicological NOAEL.

Implications of hormesis for risk assessment

Hormesis: the default model

Hormesis has important potential implications for the risk assessment process for both carcinogens and non-carcinogens. The most significant initial issue is what dose–response model should be given the designation of default. A default dose–response model would be used in the risk assessment process when toxicological data are inadequate to define confidently the dose response relationship, typically in the low dose zone.

Currently the U.S. EPA uses the threshold model as its default model for application to non-carcinogens while linear at low dose modeling is applied to carcinogens. In general, when there are only 3–4 high doses used in toxicological studies it is difficult to confidently distinguish between models in the low dose zone. That is, it is not typically possible to discern which model is best. In such instances the risk assessor selects the default dose response model. Thus, a critical decision is the selection of the default model since most carcinogen risk assessments will be based upon it.

On what basis is the default dose response model selected[64,65]? In the past, no objective criteria were established upon which this decision was based. By the time formal thinking on the matter was becoming evident the threshold model had been long established within the toxicological and regulatory communities. In the case of linearity at low dose modeling for carcinogenic risk assessment the decision was made on mechanistic (i.e., mutation) plausibility as well as guidance from a public health oriented protectionist philosophy.

It is the contention here that decisions of which model(s) become(s) the default should be based on verifiable data, using objective *a priori* evaluative methods (Table 1). As seen in the above discussion, the only model that has been successfully validated in objective evaluation has been the hormetic model. This has been achieved with data derived from the toxicological literature and with two large databases dealing with antitumor and antibiotic agents.

Table 1 Default dose–response model criteria

Generalizability by biological model, endpoint measured and chemical class/physical agent
Frequency in the toxicological literature
Application of dose–response model for endpoints of relevance to risk assessment
Capacity for false positive and negative estimates
Impact of model on hazard assessment study requirements
Capacity to estimate risk quantitatively
Ability to validate risk estimates
Capacity to assess public health implications

Furthermore, in the case of hormesis, it is independent of endpoint, including endpoints of the process of carcinogenesis, that is, initiation, promotion and progression. Since the dose response features of the hormetic dose response are the same for non-carcinogens and carcinogens, the hormetic model could harmonize risk assessment procedures for both endpoints. Most importantly, the hormetic dose response predictions can be tested and evaluated whether they involve non-carcinogens or carcinogens. This is because the hormetic stimulatory response is predicted to occur starting just below the toxicological threshold, that is, in the observable zone. This is in striking contrast to the linearity at low dose predictions that would require huge numbers of animals, making such efforts impractical as seen with the ED01 study.

Is there a need to prove hormesis in every case?

Even though there is substantial evidence to support the existence of hormesis in the toxicological literature would it have to be demonstrated in each instance where it would be implemented within a regulation. While this may be seen to be a reasonable position, it would have the net effect of never (or almost never) reaching the point whereby the hormesis concept could be implemented. The reason is that hormesis is hard to prove with limited testing. For example, there is the need to first establish a reliable NOAEL for multiple endpoints. Once this is achieved then follow up investigations should be conducted to study responses in the below NOAEL zones. If hormetic responses were observed, by definition they would be modest and would most likely require replication(s). Such studies would add considerable extra expense and time, thereby providing substantial disincentive to industry from pursuing these objectives.

The scientific foundations of hormesis as a dose response principle in toxicology has been established far beyond normal rigorous criteria for generalization across biological models, endpoints, chemical classes, and mechanisms. If it were accepted as the default model in toxicology what would be required is the estimation of a

reliable threshold via NOAEL or BMD processes. Once this is established knowledge of the quantitative features of the hormetic dose response can be applied to specific hazard assessment data.

Applying hormesis to cancer risk assessment

There are several key features of the hormetic dose response model that can affect the risk assessment process.

A Hormesis is a special type of threshold model. In the case of carcinogens the hormetic dose response model rejects the low dose linearity assumption in favor of a threshold. In this sense, the hormetic and threshold models are in agreement with the high dose threshold of the hormetic dose response being the threshold of the threshold model. As discussed above, the major interest in the hormetic model from the regulated industry perspective was to use it as a means to prove that carcinogens display thresholds and should be regulated on that basis, that is, using a set of UFs. The goal of replacing linearity at low dose modeling with a threshold default was the key objective whether it was due to an acceptance of the threshold model or the hormesis model.

B Hormesis predicts benefits at low doses. The second key feature is that the zone below the threshold in the hormetic model in cancer bioassays is predicted to reduce the risk below that observed in the control group. While trying to estimate the nadir of the J-shaped curve would appear to be an important public health-based goal it has not been an objective of private-sector organizations principally interested in lowering costs due to highly conservative carcinogen regulations based on the linear dose response model.

Estimating low dose benefits has also not been an objective of public health oriented agencies such as the EPA that have been locked into defending dose–response models such as the traditional threshold model whose low dose predictions are notoriously incorrect and linearity at low dose predictions that can not be practically tested or validated. Yet, the hormetic model is a public health oriented model since it not only may be used to reduce risks but also maximize health benefits.

Simply following past risk assessment practices which are based entirely upon a minimization rule where lower is "always safer" is predicted by the hormetic dose response model to increase risk as the dose approaches the control group, that is, once the nadir of the curve has been past.

C Hormesis assesses interindividual variation. The risk assessment can be made more realistic by integrating a consideration of normal and high risk segments of the population. Hormetic responses occur in both the normal and high risk segments of the population. The EPA has historically assumed that the high risk segment of the population would be about 10 times more susceptible than the normal segment of the population. The high risk segments of the population are typically assumed to comprise a relatively low proportion of the total population, perhaps on the order of 5–15%, although this would vary depending on the risk factor under consideration. It will not be possible to optimize the health benefits for both the normal and the high risk segments of the population to the same agent at the same dose. That is, the nadir of the curve for the normal population would be at a higher dose than for the high risk group.[66]

Under such circumstances society would have to decide whether, as a general guiding principle, it would be best to ensure the greatest good for most individuals or for the smaller number of higher risk individuals. While it is unfortunate that all cannot be equally protected/benefited, the historical answer of the EPA to have a goal of zero exposure would result in enhanced disease incidence for normal and high risk groups. As a generic approach the hormetic model offers more options and more public health benefits to the entire population including both normal and high risk groups.

D Integrating the hormetic and LNT models for cancer risk assessment. While it may appear that the LNT and hormetic dose response model yield diametrically different risk estimates in the low dose zone, it has recently been proposed that these two models could be practically integrated in cancer risk assessment. This concept is founded on the assumption that the optimized hormetic response is observed at the dose which is associated with a 10^{-4} cancer risk based on the LNT model as applied to animal cancer studies.

Since neither prediction can be proven with data from traditional chronic bioassays, it is not possible to prove or discredit either. By making the 10^{-4} risk the acceptable risk it also assures that the optimal hormetic benefit would be predicted. This approach therefore combines the predictive utility of two opposing models. This approach would likely assure that there is no risk greater than 10^{-4} while offering the theoretical upside benefits predicted by hormesis. This is a practical compromise that would bring two contrasting models together in a type of statistical ecumenism in which common ground is sought but without compromise.

Dose response latency and preconditioning

Druckery[67] published substantial experimental findings that assessed the relationship of dose to tumor latency. He noted that as the dose decreases there was an increase in tumor latency. Based on multiple experimental findings, he derived a mathematic model of the relationship between dose and tumor latency. He found that when the dose was reduced by a factor of 1000, the latency increased by a factor of 10. These findings were subsequently supported by Jones and Grendon[68] and Jones.[69] An important mechanism underlying latency period modulation is the induction of tumor promotional stimuli typically display a higher threshold than genotoxicity.[70] While Druckery and others established that latency was inversely related to dose,

Mitchell[71] has reported that latency may also be affected by the hormetic phenomenon called preconditioning. Preconditioning occurs when a prior low dose of a toxic agent/stress reduces the toxicity of a subsequent and more substantial exposure of the toxic agent(s). Mitchell[71] reported that a prior low dose exposure of gamma rays (10 mGy) significantly increased the latency of lymphoma in a cancer prone mouse model given a single carcinogenic dose of 4 Gy. The prior dose extended the latency by about 100 days whereas a 10-fold higher preconditioning dose did not alter the tumor latency.

Despite the fact that tumor latency modulation by dose has not been integrated into the current cancer risk assessment paradigm this concept is worthy of considerable future consideration as it offers the potential to derive thresholds for carcinogens (i.e., that is, at a low dose, the latency might extend far beyond the estimated normal maximum lifespan of humans). In fact, the manner in which Druckery proposed the dose latency relationship was based upon an LNT framework. The research of Mitchell[71] also showed that it is also compatible with an hormetic framework.

Applying hormesis to non-carcinogen risk assessment

The principal impact that hormesis would have on non-carcinogen risk assessment would be its effect on the magnitude of the current inter-individual uncertainty factor. In its routine procedures the EPA utilizes two UFs in estimating possible human responses based on animal model studies. These include the animal to human and the inter-individual variation UFs. Since both factors are assumed to be 10-fold and independent of each other, they are multiplied yielding a 100-fold total uncertainty factor that is used to reduce human exposures when based on animal model studies. If one were to follow the EPA uncertainty factor approach it would invariably result in a reference dose (RFD) value that would offer no hormetic benefit to either the normal or high risk segments of the population.[66] In order to benefit either group the inter-individual uncertainty factor of 10 would have to be either decreased to 5-fold in order to be in the hormetic zone for the normal segment of the population, or increased by 20-fold in order to be in the hormetic zone for the generic high risk segment of the population.

Concerns with hormesis

The principal concern with the hormesis is the challenge that it presents to the toxicology community to better understand the entire dose–response continuum rather than being content with the assumption that the dose–response starts as it exceeds the NOAEL. While the costs of accepting the challenges that hormesis creates can be formidable, the need for many additional doses and time points for evaluation can be at least partially compensated by the use of alternative biological models which are considerably less expensive such as cell culture as well as more integrated use of plant, microbial, invertebrate and fish models in overall hazard assessment strategies.

An issue with hormesis is the response can be beneficial, neutral or harmful. In the case of a harmful effect, this could mean that any change from control would be a concern. For example, if an agent caused the prostate gland to decrease in size at high doses but increase in size at low doses as to be clinically important then either change would raise concerns. The traditional NOAEL would not be an effective basis upon which to establish a safe level. The maximum stimulation in the low dose or hormetic zone is typically only 30–60% greater than the control. Whether changes of this magnitude in the parameters of interest presents clinical/public health implications would have to be assessed on an endpoint by endpoint basis. However, if the changes were deemed of public health or clinical significance then it may be necessary to utilize the lower dose threshold of the hormetic dose response for risk assessment proposes. The width of the stimulatory zone would also be of interest since it has the capacity to be variable. This is an important and poorly understood area since the width could vary over a considerable range with clinical, public health and economic implications.

Hormesis and the issue of chemical mixtures

The area of multiple chemical exposures has often been raised with respect to hormesis and how it would affect hormetic predictions. Mixture toxicology is a significant challenge within toxicology and its lack of understanding is not unique to the issue of hormesis. However, the research[72–75] concerning memory indicates that synergistic effects may be commonly observed with agents that by themselves cause hormetic dose responses. The critical observation in these cases was that the interaction of two agents in which both caused an hormetic effect with respect to learning frequently displayed a synergy at low doses. However, the synergistic interaction did not result in the response being significantly greater than that observed by the individual hormetically acting agents at higher doses. The 30–60% increase in learning was achieved via synergy with doses markedly lower than achieved by a single agent. That is, the concept of synergy with respect to hormesis was not with the size of the effect which seemed to be capped by the biological constraints imposed by the hormetic process, but with the size of the dose to achieve the capped or maximum performance. This is a novel concept in toxicology and one that has yet to be appreciated within the toxicological literature.

Hormetic effects may occur at the same dose at which a toxic effect occurs

Toxic substances may cause adverse effects in multiple tissues but at different doses. This suggests that each adverse response will have its own unique dose–response

relationship. In hazard assessment evaluation with a three or four dose experiment it is possible that an hormetic response for one endpoint may occur at the lower two doses, or at doses below those tested, while another effect may display toxicity at the same lower two doses. In such cases how would the risk assessment process address the issue of hormesis? The risk assessment process requires that a NOAEL should be derived for all endpoints measured. If the hormetic dose response relationship were used as the default then it would be necessary to either construct from data or to estimate the dose response relationship for each endpoint. Once this information is assembled it would then have to be evaluated within the context of a public health assessment. This would take into account the nature of the endpoints measured, their public health implications and the capacity of the experimental systems used to extrapolate the effects to humans.

Summary

1 The threshold dose response model, which has been the principal model used in toxicology, pharmacology and clinical medicine for the past century, provides unreliable estimates of response to doses below the traditional toxicological threshold effect. It should not be used as the default model in risk assessment practices designed to estimate low dose treatment effects.

2 Rejection of the use of the threshold model in the risk assessment process is significant because large numbers of regulatory decisions affecting the public health, patient health in clinical settings, and environmental receptors have been based on the application of the threshold model.

3 The continued use of linearity at low dose modeling as applied in the risk assessment of carcinogens is also problematic since such estimates can not be practically assessed or validated.

4 The hormesis model has been shown to be more common than any other dose response model, far out performing its competitor models such as the threshold and linear at low dose models.

5 The hormetic model predictions are generalizable, without restriction to biological model, endpoint measured, chemical class, and mechanism.

6 The hormetic model has been ignored and/or rejected in the past, principally because the field of toxicology adopted the threshold dose response model without validation and developed its entire testing and evaluation programs for chemicals and pharmaceutical agents on the belief that it was only necessary to test with a few doses at high levels to estimate LOAELs and NOAELs.

7 The adoption of the hormetic model for the purposes of risk assessment represents a significant advance in the evaluation process. Most importantly, all hormetic predictions can be validated and either accepted or rejected, something that linearity at low dose assumptions can not offer.

Second, if hormesis were accepted as the default model in risk assessment it would not be necessary to change the hazard assessment process since below threshold effects can be reliably estimated once the NOAEL is obtained. The hormetic model provides the risk assessor with the opportunity for the first time to not only provide a means to reduce harm to the general public but to also maximize potential health benefits as predicted by the hormetic model.

8 The hormetic model provides the capacity to estimate health hazards to the public when both above and below threshold effects may have the potential to cause adverse health effects. The current threshold model only recognizes the potential for adverse effects at doses exceeding the threshold.

9 The adoption of the hormetic dose response model as the default in risk assessment is supported by a strong preponderance of the scientific evidence.

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