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Environmental Toxicology and Chemistry, Vol. 27, No. 7, pp. 1451–1474, 2008

Printed in the USA
0730-7268/08 $12.00 + .00

Critical Review

HORMESIS: WHY IT IS IMPORTANT TO TOXICOLOGY AND TOXICOLOGISTS

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(*Received* 11 *October* 2007; *Accepted* 24 *January* 2008)

Abstract—This article provides a comprehensive review of hormesis, a dose–response concept that is characterized by a low-dose stimulation and a high-dose inhibition. The article traces the historical foundations of hormesis, its quantitative features and mechanistic foundations, and its risk assessment implications. The article indicates that the hormetic dose response is the most fundamental dose response, significantly outcompeting other leading dose–response models in large-scale, head-to-head evaluations. The hormetic dose response is highly generalizable, being independent of biological model, endpoint measured, chemical class, and interindividual variability. Hormesis also provides a framework for the study and assessment of chemical mixtures, incorporating the concept of additivity and synergism. Because the hormetic biphasic dose response represents a general pattern of biological responsiveness, it is expected that it will become progressively more significant within toxicological evaluation and risk assessment practices as well as have numerous biomedical applications.

Keywords—Hormesis Biphasic U-shaped J-shaped Dose response

INTRODUCTION

The present paper provides a comprehensive review of hormesis, a dose–response model that has come to be more broadly and consistently observed as toxicologists and pharmacologists direct their efforts to explore possible responses in the low-dose range. The investigation of low-dose effects has begun to transform toxicology from a discipline dominated by high doses to one that explores toxic mechanisms and underlying adaptive responses. In doing so, this new toxicology is revealing biological processes and mechanisms that become manifest only at low dose and/or are obscured by the traditional high-dose paradigm that has been dominant for so long in the field. So significant have these research advances in the low-dose domain become that they can alter how hazard assessments are conducted, risk assessments are practiced, drugs are designed and tested, and patient doses are optimized.

The present paper is organized by the framing of several dozen questions that follow a progressive sequence, each with a referenced-based, documented response. The series of questions and responses are designed to lead to the final question that also is the title of this article. The interested reader also may find the following major reviews of interest [1–5]. To provide an integrative summary of the subsequent sections, Appendices 1 through 3 list the key principles underlying hormesis (Appendix 1), the observations that support these principles (Appendix 2), and the implications of the hormetic principles for toxicology/risk assessment and clinical practice (Appendix 3).

WHAT IS HORMESIS?

Hormesis is a biphasic dose–response phenomenon characterized by a low-dose stimulation and a high-dose inhibition [1,6,7]. Hormesis is a special type of biphasic dose–response relationship that has well-defined, quantitative features, including the magnitude and the width of the stimulatory zone and the relationship of the stimulatory zone to the traditional toxicological threshold (no-observed-adverse-effect level) and, in certain features, its equivalent called the zero equivalent point (Fig. 1). The hormetic dose response also must be seen within a temporal context—that is, as a dose–time–response relationship. The reason for incorporating a temporal feature in hormesis is that it also may be described as a modest overcompensation response following an initial disruption in homeostasis—that is, a type of rebound effect (Fig. 2). The hormetic dose response therefore represents the effects of a reparative process that slightly or modestly overshoots the original homeostatic set point, resulting in the low-dose stimulatory response [8,9]. Figure 3 provides a representative selection of hormetic dose responses, reflecting its occurrence across a broad range of biological models, endpoints, and chemical agents.

The assessment of the dose response therefore is a dynamic process. Whereas harmful agents may induce toxicity in affected biological systems, the organism or biological system is not a passive entity but, rather, will respond to damage signals with a coordinated series of temporally mediated repair processes. This dynamic aspect of toxicological assessment requires the inclusion of not only a broad range of doses but also a series of temporal evaluations (i.e., repeat measures). Only by assessing the dose–response process over time can an accurate assessment of the dose–response relationship be determined, within which the hormetic dose response is best revealed. Toxicological assessments that include either too few doses, too high doses, inadequate dose spacing, or only one time point for evaluation are not capable of accurately assessing the nature of the dose–response relationship.

Hormesis therefore is more than simply a dose–response relationship or a dose–time–response relationship but, rather, a quantitative manifestation of a reparative process that is adaptive in nature. The modest nature of the low-dose stimulation reflects the capacity of the biological system to allocate

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Published on the Web 2/14/2008.

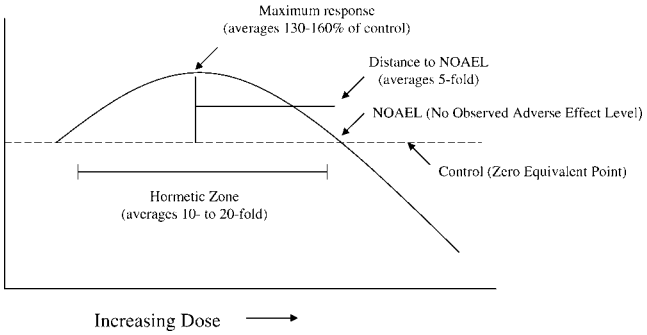


Fig. 1. Dose–response curve depicting the quantitative feature of hormesis [10].

biological resources in a highly efficient manner during the reparative process. That is, if the goal is to reestablish the homeostatic condition, it would make little sense to induce an overcompensation stimulation in the several-fold or more range, because that would be wasteful of biological resources. However, it would be important to reestablish homeostasis as efficiently as possible after injury. Thus, the overcompensation stimulation is modest, being only in the percentage (not fold) zone, with a maximum usually being only approximately 30 to 60% greater than that seen in the controls [7,10]. Whereas this modest overcompensation response has been highly conserved in an evolutionary sense, it often makes it difficult for toxicologists to assess hormesis, because it may be hard to detect, especially when study designs have too few doses, limited statistical power, and only one time point. Thus, the study of hormesis places greater resource and time demands on investigators.

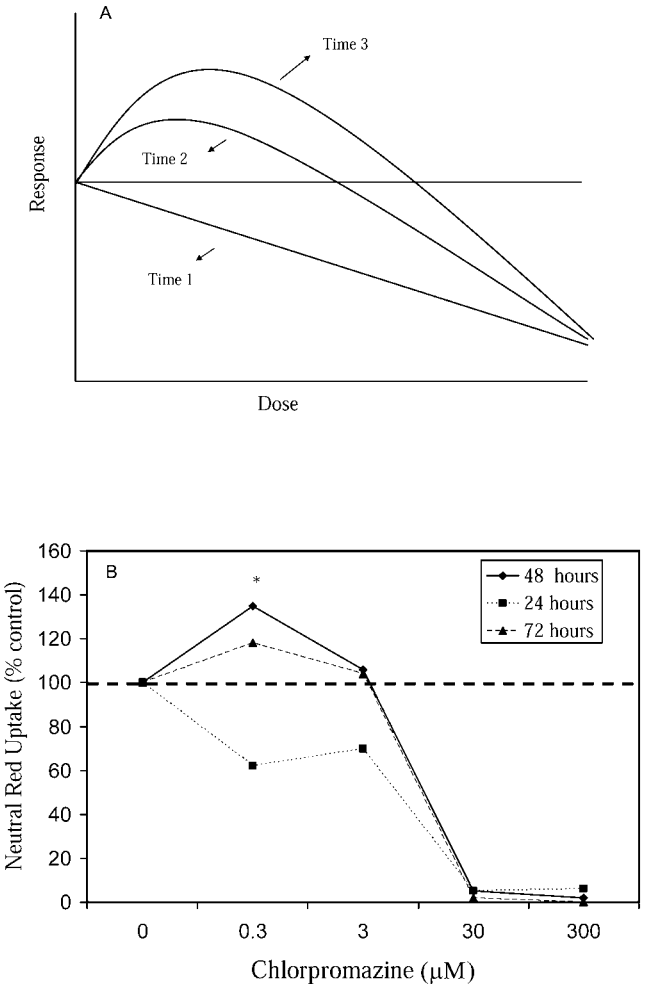


Fig. 2. Hormetic dose–time–response relationship: Modest overcompensation following a disruption in homeostasis (for review, see [8,167]).

WHO DISCOVERED HORMESIS?

It is widely believed that Hugo Schulz, a professor of pharmacology at the University of Greifswald in northern Germany, discovered the concept of hormesis during the middle portion of the 1880s. The results of the discovery were published in the late 1880s in several papers [11,12] that assessed the effects of various disinfectants on the metabolism of yeast. In his investigations, Schulz reported that numerous toxic agents stimulated the release of carbon dioxide at low concentrations while being inhibitory at higher doses. In an autobiographic statement on the occasion of his 70th birthday, Schulz recounted the moment of the discovery (see Crump [13], English translation of the 1923 statement of Schulz):

> Since it could be foreseen that experiments on fermentation and putrescence in an institute of pathology would offer particularly good prospects for vigorous growth, I occupied myself as well as possible, in accordance with the state of our knowledge at the time, with this area. Sometimes, when working with substances that needed to be examined for their effectiveness in comparison to the inducers of yeast fermentation, initially working together with my assistant, Gottfried Hoffmann, I found in formic acid and also in other substances the marvelous occurrence that if I got below their indifference point, i.e., if, for example, I worked with less formic acid than was required in order to halt the appearance of its antifermentive property, that all at once the carbon dioxide production became distinctly higher than in the controls processed without the formic acid addition. I first thought, as is obvious, that there had been some kind of experimental or observation error. But the appearance of the overproduction continually repeated itself under the same conditions. First I did not know how to deal with it, and in any event at that time still did not realize that I had experimentally proved the first theorem of Arndt's fundamental law of biology.

It was quite obvious that the low-dose stimulation was completely unexpected, forcing Schulz and his assistant to repeat their experiments until they were satisfied that the phenomenon was reproducible.

Whereas this research was the key discovery of Schulz and the papers that set the concept of hormesis in motion, it was Schulz's proclamation that his findings provided the explanatory principle of the medical practice of homeopathy—and his long-term and highly visible commitment to this perspective—that raised Schulz to the level of historical figure and creator of the hormesis concept. In fact, a recent paper by Henschler [14] indicated that the earliest discoverer of the hormetic concept may have been the famous scientist Rudolph L.K. Verchow, based on work published in 1854. It was Schulz's linkage of this concept to the controversial medical practice of homeopathy that made him well known, but because of political/ideological perspectives, this also created enormous difficulties for this fledgling dose–response theory to get a fair scientific hearing within the confines of traditional medicine and its subsequent spin-off disciples, such as pharmacology and toxicology and even, far later, risk assessment. Surprisingly, despite his controversial standing in the biomedical sciences, Hugo Schulz was nominated in 1931 for the

Nobel Prize based on his original 1887/1888 publications (http://www.nobelprize.org/medicine). Schulz died a year later.

WHEN WAS THE TERM HORMESIS CREATED?

The term hormesis was first published in the open literature in 1943 by Chester Southam, a graduate student in forestry at the University of Idaho, and John Ehrlich, the advisor of Southam [15]. The paper assessed the effects of extracts from the red cider plant on the metabolism of multiple fungal species, showing a low-dose stimulation and a high-dose inhibition (Fig. 4). Whereas 1943 is the official date for the creation of the term hormesis, a more careful look reveals that Southam actually first employed the term in his 1941 undergraduate thesis. In this thesis, Southam acknowledged the occurrence of biphasic dose responses in bacterial studies but did not cite any references. A copy of this undergraduate thesis has been obtained and is now available online (http://www.dose-response.org).

Many terms have been—and currently are being—used for what appears to be the dose–response relationship, which is called hormesis. Some of these terms include biphasic, nonmonotonic, bell-shaped, U-shaped, inverted U-shaped, J-shaped, overshoot, rebound effect, bitonic, functional antagonism, preconditioning, and adaptive response. Other terms have been used that indicate this phenomenon has been considered to be the equivalent of a biological law. This is seen with the terms Yerkes-Dodson law [16], Hueppe's Rule [17], and the Arndt-Schulz law [18–20], named after the original formulators of the concept.

The use of such a variety of terms for the same or closely related dose–response phenomena may seem to be unusual. Often, however, these terms are specific to a given biological subdiscipline. For example, the Yerkes-Dodson law is employed exclusively in the area of psychology [21]. Overshoot and rebound effects typically are used in disciplines in which initial toxicity because of dose treatment is expected. These terms can be seen in the areas of cancer chemotherapy [22] and animal herbivory [23], in which responses to damage are the key biological endpoints measured. Functional antagonism is used almost exclusively in the field of pharmacology [24]. Some terms are employed more generally, such as U-shaped, but nonetheless are used extensively in some disciplines, such as epidemiology [25–28].

The use of many terms for the same concept is principally the result of the high degree of disciplinary specialization and inadequate communication between the subdisciplines. Thus, terminological divergence and concept confusion on the nature of the dose response is, in large part, a result of the overly domineering tendency toward specialization within the biological sciences [29].

IS HORMESIS THE BEST TERM?

Hormesis may be the most appropriate term because of its long history in the published literature [15] and its more than 800 citations in the *Web of Science*® (http://www.thomsonscientific.com/) as of 2007. In addition, hormesis represents a very specific type of biphasic dose–response relationship with quantifiable dose–response features that are highly generalizable, are specific temporal features, and have a definitive relationship to the toxicological threshold—features generally lacking in other possible terms. The issue of biological stress terminology is now recognized as a serious one within the biological and biomedical sciences. Recent efforts have been made to establish a common terminology for stress-related dose–response relationships [29] that are capable of integrating diverse interdisciplinary perspectives on the nature of the dose response in the low-dose zone (Fig. 5).

DOES HORMESIS IMPLY A BENEFICIAL RESPONSE?

In 2002, Calabrese and Baldwin [30] published a paper entitled ''Defining hormesis'' in which they argued that hormesis is a dose–response relationship with specific quantitative and temporal characteristics. It was further argued that the concept of benefit or harm should be decoupled from that definition. To fail to do so has the potential of politicizing the scientific evaluation of the dose–response relationship, especially in the area of risk assessment [31–33]. Calabrese and Baldwin also recognized that benefit or harm had the distinct potential to be seen from specific points of view. For example, in a highly heterogeneous population with considerable interindividual variation, a beneficial dose for one subgroup may be a harmful dose for another subgroup (Fig. 6). In addition, it is now known that low doses of antiviral, antibacterial, and antitumor drugs (Fig. 7) [2] can enhance the growth of these potentially harmful agents (i.e., viruses), cells, and organisms while possibly harming the human patient receiving the drug. In such cases, a low concentration of these agents may be hormetic for the disease-causing organisms but harmful to people. In many assessments of immune responses, it was determined that approximately 80% of the reported hormetic responses that were assessed with respect to clinical implications were thought to be beneficial to humans (Appendix 4). This suggested, however, that approximately 20% of the hormetic-like low-dose stimulatory responses may be potentially adverse [3]. Most antianxiety drugs at low doses display hormetic dose–response relationships, thereby showing beneficial responses to animal models (Fig. 8) and human subjects. Some antianxiety drugs enhance anxiety in the low-dose stimulatory zone while decreasing anxiety at higher inhibitory doses. In these two cases, the hormetic stimulation is either decreasing or increasing anxiety, depending on the agent and the animal model [34]. Thus, the concepts of beneficial or harmful are important to apply to dose–response relationships and need to be seen within a broad biological, clinical, and societal context. The dose–response relationship itself, however, should be seen in a manner that is distinct from these necessary and yet subsequent applications.

DEFINING THE QUANTITATIVE FEATURES OF THE HORMETIC DOSE RESPONSE

When the hormesis database was created in the mid-1990s, only a limited understanding existed regarding what, if any, general quantitative features of the hormetic dose response might exist [7]. The hormesis database, however, included information concerning the maximum stimulation, the width of the stimulatory response, and the width or distance from the peak of the stimulatory response to the estimated zero equivalent point [7]. Based on the analysis of thousands of dose–response relationships with evidence of hormesis, it became clear that the maximum stimulatory response of hormetic dose responses was modest—usually not exceeding the control value by more than twofold. In fact, the maximum stimulatory response generally was only approximately 30 to 60% greater than the control value (Fig. 1) [10]. This was the case regardless of the biological model studied, the endpoint measured, and the chemical or physical agent tested. The maximum

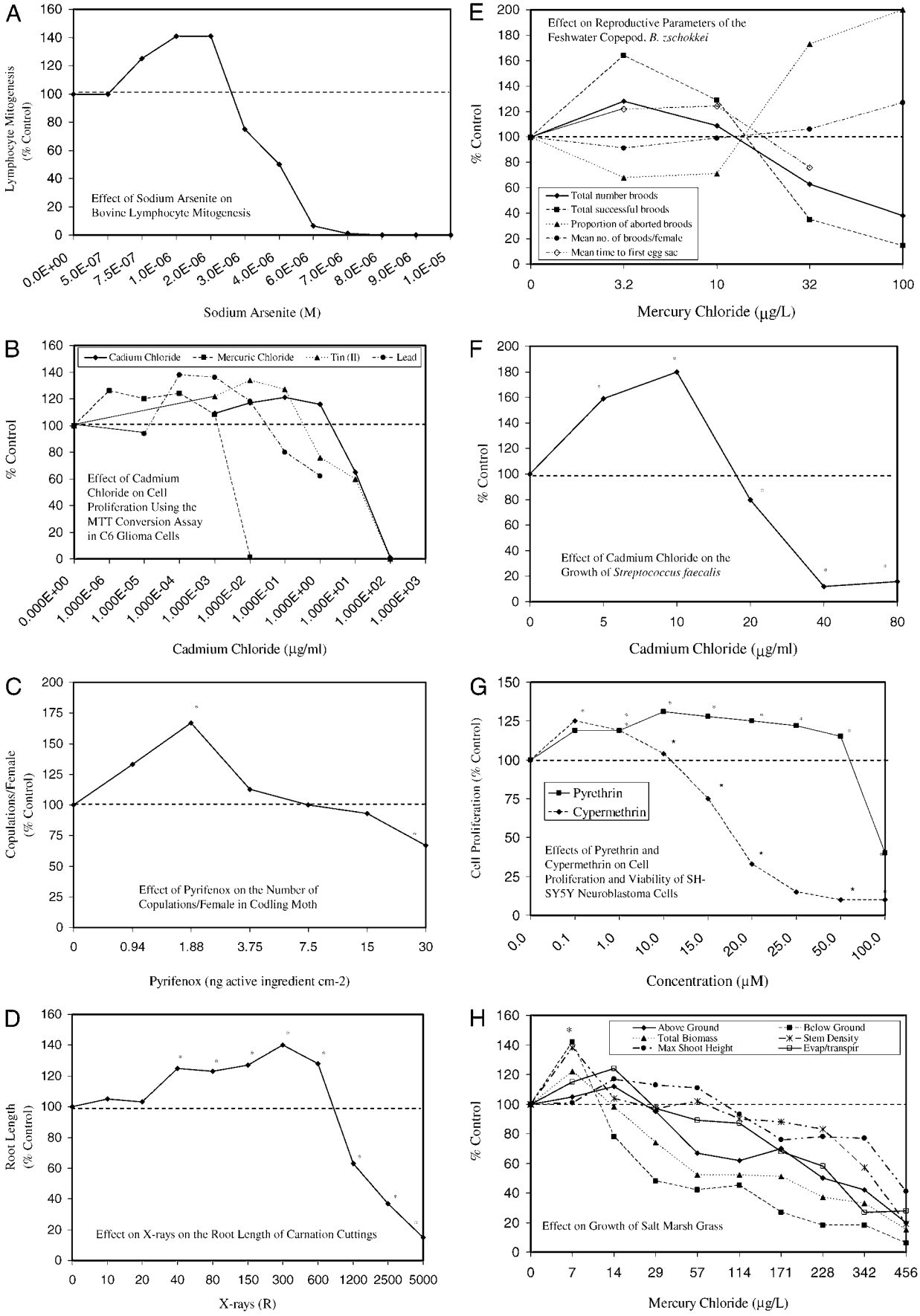
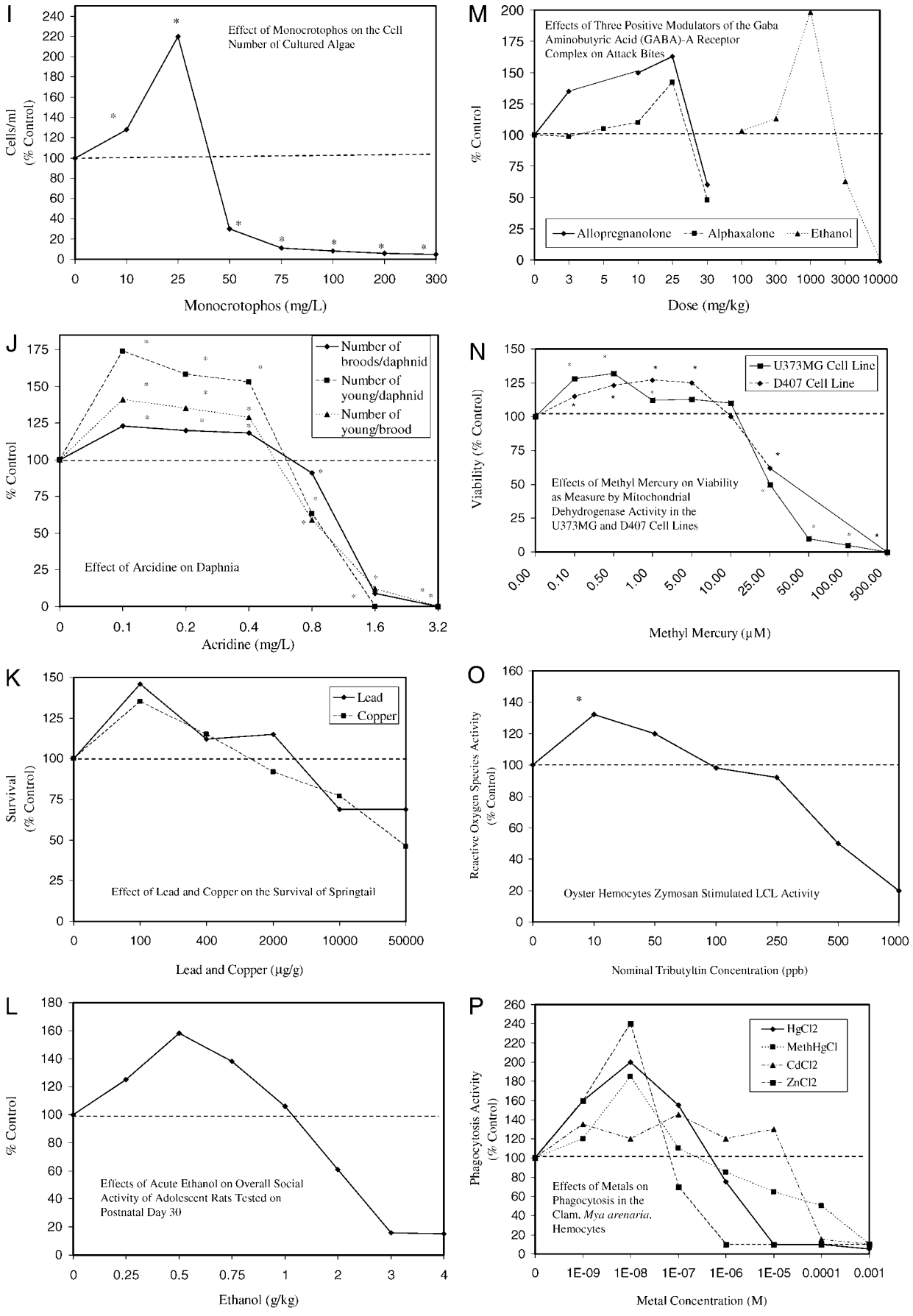


Fig. 3. Multiple hormetic responses [168–184]. Asterisks denote statistical significance ($p \leq 0.05$).



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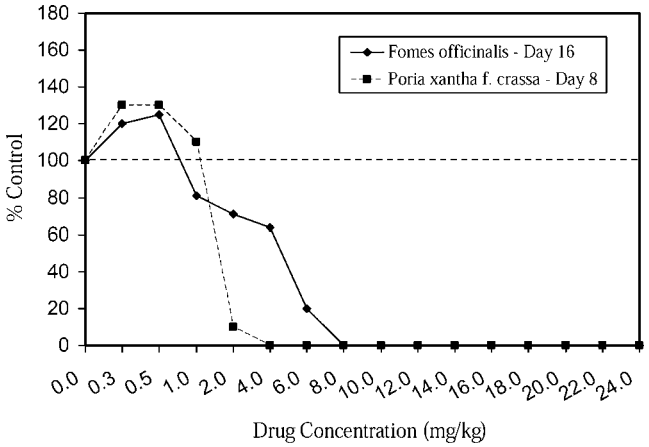


Fig. 4. Percentage of normal growth on malt agar containing various concentrations of western red-cedar heartwood extract on selected fungal species [15].

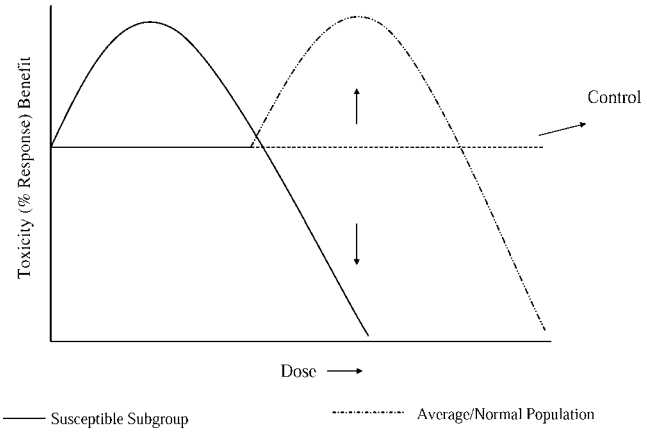


Fig. 6. Hormesis and differential susceptibility.

stimulatory response has become the most distinguishing characteristic of the hormetic dose–response relationship. In contrast to the maximum stimulatory response, the width of the stimulation has been more variable. The vast majority of the widths of the stimulation are less than 100-fold, but approximately 2% of the dose responses in the database have a stimulatory width that exceeds 1,000-fold (Fig. 9). The reasons for the variability in the width of the stimulatory zone are uncertain. It is quite likely, however, that the more homogenous the sample population, the less variable the width of the stimulation range.

DOES THE MAGNITUDE OF HORMETIC STIMULATION VARY?

Approximately 10 to 20% of the dose responses in the hormesis database have maximum stimulatory responses that approach or exceed by twofold that reported in the control group. It is not clear if this is normal variation, measurement error, species-specific responses, or some other factor. It is possible that such higher-than-expected stimulatory responses might represent a breakdown in the regulatory control procedures that control biological resource allocation. This might be expected to occur in aged members of the population. However, these suggestions have yet to be studied.

Based on the above discussion, the question must be raised as to how biphasic dose responses would be classified if a 5- to 10-fold stimulation existed in the low-dose range and inhibition at the highest dose. Would this more extreme example of a low-dose stimulation still be considered an example of hormesis? This question has no clear answer. The vast majority of biphasic dose responses do not show such a large stimulatory response. Because this is the case, it would be necessary first to assess the reproducibility of the observation (i.e., whether the control group was aberrantly low). There may

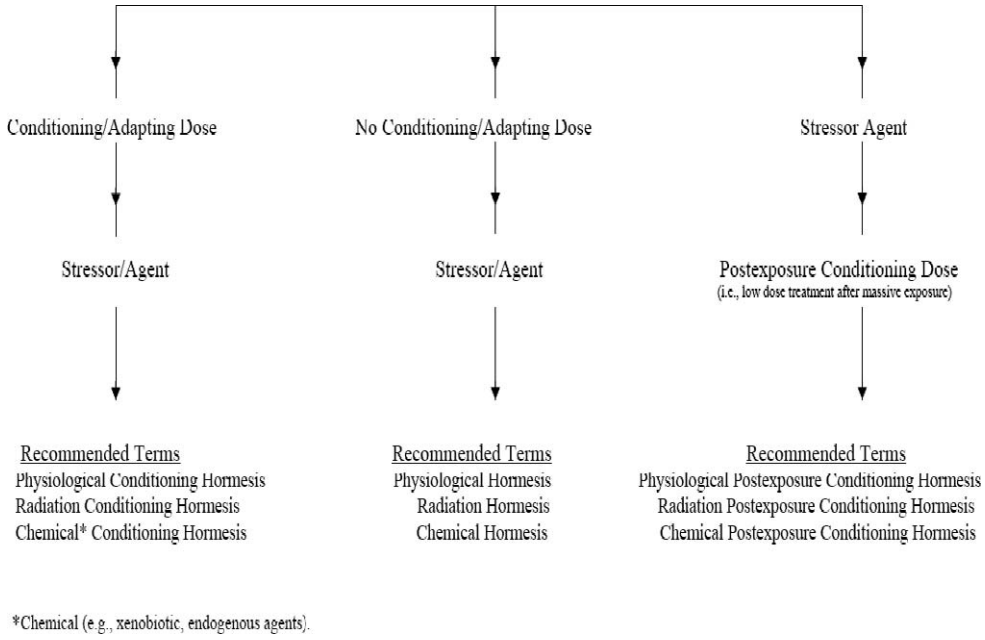


Fig. 5. Summary of biological stress terminology. Preconditioning, adaptive response, and autoprotection represent examples of what is described here as conditioning hormesis. Advantages include the following: Standardized terminology provides information regarding the presence or absence of a conditioning dose, whether it is before or after the more massive challenge, and the nature of the stressor agents. This terminology would establish a consistent and understandable framework across the spectrum of biological disciplines concerning dose–response and stress–response relationships [29].

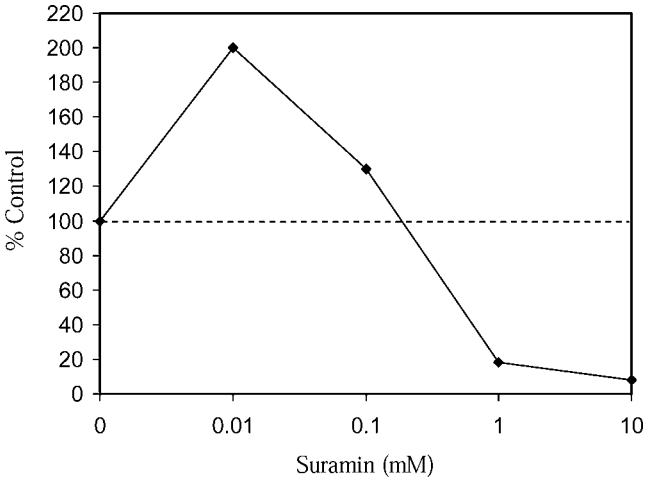


Fig. 7. Effect on prostate cancer cell (MLL) growth [2,184].

well be a set of biphasic dose responses, however, with markedly different quantitative features that has not yet been investigated.

CAN A MODEST STIMULATORY RESPONSE BE CONSIDERED A REAL HORMETIC EFFECT?

If a response were quite modest—that is, less than 10% greater than the control—the findings would have to be assessed in well-designed studies with excellent statistical power and be properly replicated. Nonetheless, the fact that an increase is quite modest should not be grounds for assuming it is simply background variation and lacking in biological or even economic significance. For example, a very small but consistent increase in body weight for farm animals, such as poultry, could have very notable economic impacts [35].

IS A U-SHAPED DOSE RESPONSE WITHOUT TOXICITY/INHIBITION A HORMETIC DOSE RESPONSE?

In theory, a dose response that shows only a low-dose stimulation without a high-dose inhibition (i.e., a pure U-shaped dose response) does not satisfy the quantitative features of a hormetic dose response. Because, however, possible limitations in study designs include an inadequate number of doses, variability concerns, heterogeneity of the subjects tested, need for replication, and temporal components, it can be difficult to conclude with confidence that a purely U-shaped dose response, with no toxicity/inhibition at higher doses, exists. Furthermore, it is possible that a broad family of hormetic-like dose responses with various modifications in the quantitative features of the dose response may exist. If such were the case, then one could consider the need for testing, detection, classification, and assessment for the respective biological implications for the range of possible U-shaped dose–response relationships.

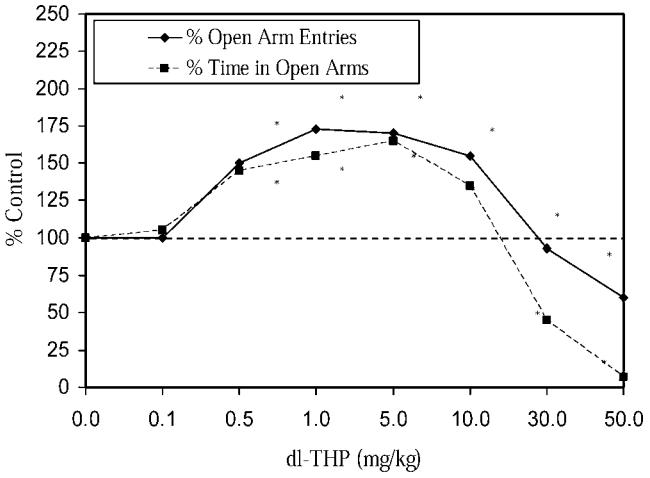


Fig. 8. Anxiolytic effect of dextrorotatory levorotatory–tetrahydropalmatine (DL-THP), a naturally occurring alkaloid, on Institute of Cancer Research (ICR) mice of both sexes in the elevated plus-maze test. An asterisk indicates a significant difference from the control ($p < 0.05$) [21,185].

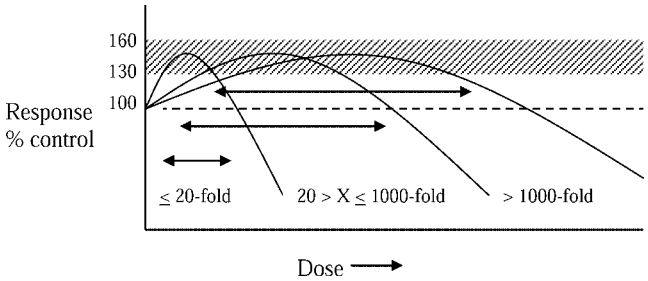


Fig. 9. Dose–response curves indicating the relative distribution of stimulatory dose ranges. The shaded area represents the maximum stimulatory response range, which typically is 130 to 160% of the control value.

WHAT IS THE DIFFERENCE BETWEEN DIRECT STIMULATION AND OVERCOMPENSATION HORMESIS?

Whereas hormesis has been defined as a dose–response relationship that is characterized by a low-dose stimulation and a higher-dose inhibition within the context of an overcompensation framework, considerable data indicate that biphasic dose responses may occur within a direct stimulation experimental framework. Under most circumstances in the published literature dealing with dose–response relationships, it is not possible to distinguish between a hormetic-like dose response that has resulted from an overcompensation or a direct stimulation. This is because approximately 75% of the studies that demonstrate hormesis have only included measurements at one time point [10]. For the vast majority of the thousands of examples of hormesis in the published literature, no judgment therefore can be rendered on which specific type of hormesis is present. Nonetheless, enough evidence exists to document that both overcompensation and direct stimulation types of hormesis exist. These quantitative features may be similar because they are carrying out biological functions within similar plasticity constraints, thereby leading to quantitatively comparable quantitative features of the dose response.

DOES A BIOLOGICAL SYSTEM HAVE TO BE STRESSED/DAMAGED TO EXHIBIT HORMESIS?

If direct stimulation hormesis is induced [30], then the answer is no. Although speculative, it may be possible that a hormesis-inducing agent could bypass a toxicity mechanism and act at a downstream mechanism to induce a hormetic response. This represents a therapeutic possibility yet to be demonstrated.

WHAT ARE THE SURVIVAL ADVANTAGES OF HORMESIS?

At least four major features of the hormetic response would enhance survival of the individual. These include direct benefit

by endogenous and/or exogenous agents, including endogenous and synthetic agonists. Recognition of these beneficial effects have led to numerous pharmaceutical applications (e.g., anxiolytic, antiseizure, and memory drugs) based on the hormetic dose response, enhanced resource allocation efficiency, functioning as a conditioning stimulus to either protect against damage from a subsequent life-threatening exposure (preconditioning hormesis) or to enhance protection/repair following a life-threatening exposure (postconditioning hormesis), and reducing the occurrence of possible endogenous agonist side effects and to increase the optimal dose–response range of endogenous agonists.

Direct benefit

Numerous examples of a dose-induced improvement exist within the context of a hormetic dose–response relationship. Some examples include memory enhancement, anxiety reduction, seizure threshold increase, bone strengthening, tumor reduction, and protection against agonists inducing neuronal diseases, such as Alzheimer's, Parkinson's, and others.

Resource allocation

In the case of resource allocation, it was mentioned previously that the goal of tissue repair would be to reestablish homeostasis as soon as possible following damage. In this case, it would be an advantage to ensure that reestablishment of homeostasis was ensured by only slightly overshooting the mark. This would provide a quick, timely, and full repair with little misallocation of biological resources. Overshooting the mark by several-fold or more would reflect inefficient control over resource allocation and, eventually, place the individual at enhanced risk.

Conditioning

In the case of protection from injury, a previous low-dose exposure may induce an adaptive response that remains active for multiple days to, possibly, more than a week, thereby providing protection against a subsequent life-threatening exposure to a massive dose and accelerating tissue repair processes [21,29]. Such adapting doses permit the organism to continue to be mobile within highly heterogeneous environments. Because the length of protection is for a limited time period, it also permits flexibility and control of the allocation of resources needed to sustain the enhanced protection. A similar but less well-studied process exists if the massive exposure occurs before the conditioning doses (i.e., postconditioning). In this postconditioning dose framework, a low dose received after the massive injury induces repair processes that result in a protective response of a magnitude similar to that seen with preconditioning exposure [36–40].

Side effects

In the case of endogenous agonists, it has been proposed that side effects occur far less frequently with partial agonists/partial antagonists as compared to full agonists [41]. The U-shaped dose response also provides a broader concentration range within which the agent may act, thereby enhancing a functional capacity with reduced risk of side effects. Because side effects of agents that act via receptor-based mechanism can be highly debilitating, their elimination/prevention can have considerable survival advantage [41].

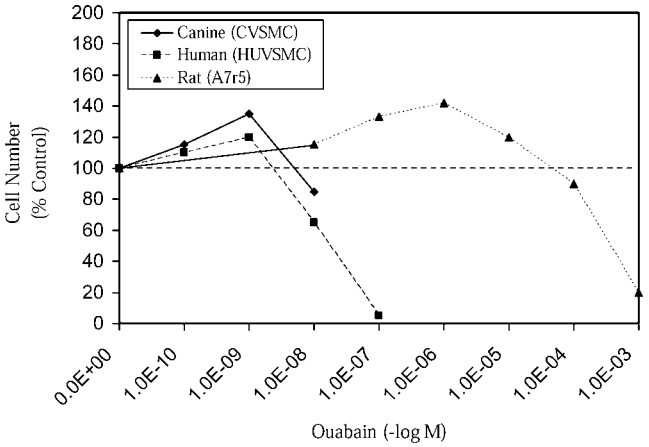


Fig. 10. Effects of ouabain on proliferation [44].

IS THERE A HORMESIS GENE AND BIOMARKER?

It is highly unlikely that one specific type of hormesis gene accounts for the wide range of specific hormetic effects reported. This is because hormetic effects occur in essentially all plant, microbial, and animal species, affecting many hundreds of endpoints in numerous cell types and tissues, involving many hundreds of genes for each endpoint. The hormetic response represents a very basic and general strategy that occurs in all types of cells and tissues using a wide variety of integrative mechanisms.

IS HORMESIS EXPECTED TO OCCUR IN ESSENTIALLY ALL PLANT AND ANIMAL SPECIES?

This is likely to be the case, because the current hormesis data set of nearly 8,000 dose responses indicates that hormetic dose responses occur in several hundred plant species as well as in numerous microorganism and animal species [1,10,42]. Given this type of broad diversity of hormetic responses and the fact that hormesis serves a series of strong survival interests, it is likely to be a universal or near-universal phenomenon.

ARE THERE INTERSPECIES DIFFERENCES IN HORMETIC RESPONSES?

Ouabain is a cardiotonic agent initially found in the ripe seeds of the African plants *Strophanthus gratus* and *Acokanthera ouabaio*. Ouabain is well known for its capacity to inhibit the enzyme Na^+/K^+-dependent adenosine triphosphatase (Na^+/K^+-ATPase) sodium pump, a feature that was exploited clinically in the treatment of congestive heart failure. During the early 1990s, ouabain also was reported to be an endogenous hormone, being synthesized in the adrenal glands, hypothalamus, and heart, with production being increased under oxygen deficiency.

Ouabain has long been exploited for its inhibitory effects at high doses, but it only recently has been recognized that at low concentrations, ouabain has the opposite effect—that is, stimulation of Na^+/K^+-ATPase [43]. This observation has been generalized and applied in a hormetic dose–response evaluation. In that study, Abramowitz et al. [44] compared the growth-promoting effects of ouabain in cultured cells from vascular smooth muscle cells from the rat, dog, and human. Following a 5-d incubation, ouabain induced a biphasic dose response in the vascular smooth muscle cells of each species. As seen in Figure 10, the human and dog cells were quantitatively similar in responsiveness while being approximately

1,000-fold more sensitive than the rat cells. Despite the greater sensitivity of the human and dog cells, the quantitative features of the dose responses were similar. It is of interest that those findings were fully consistent with those of earlier investigations indicating a three-orders-of-magnitude difference in the affinities of the sodium-pump A1 subunit of Na^+/K^+-ATPase for ouabain in the rat as compared with that for the human and dog A1 subunits [45]. Such observations support the hypothesis the ouabain-induced proliferative effects probably are mediated by its binding to the A1 subunit of the sodium pump.

The low-dose stimulatory response therefore most likely was initiated by a drug interaction with the sodium pump, as reflected by the respective affinities of the steroid for the pump-based protein A1 subunit, yet at concentrations that did not affect cytoplasmic ion levels. The basis of the interspecies difference was directly related to the well-known difference in affinity between rat and other mammalian species for ouabain and the A1 subunit of Na^+/K^+-ATPase. Whereas this interspecies comparison displays profound quantitative differences in ouabain potency between the rat and the dog/human models, the magnitude and width of the stimulatory responses were similar. This type of responsiveness would be expected, being consistent with the vast range of findings in the hormesis database [10].

The value of the above research with ouabain is that its underlying mechanistic foundations are well defined within the three experimental models, permitting insights regarding the reasons for the occurrence of the interspecies variation in potency. The mechanism-based research did not, however, provide insight concerning the quantitative features of the dose response (i.e., maximum stimulation and width of the stimulatory response). Other possible interspecies comparisons would be expected to potentially differ principally with respect to potency of the inducing agent but not with respect to the quantitative features of the hormetic dose response, especially with respect to the maximum stimulatory response, which would be independent of biological model.

Hormetic dose responses generally are believed to be independent of biological model, which suggests that the findings should be reliably extrapolated to other similar species/strains given similar testing protocols. Whereas there may be marked differences in inherent toxic susceptibilities among species, the expectation would still be that the hormetic dose response would occur across species/strains. The example selected above illustrates that the human and dog models were 1,000-fold more sensitive than the rat model, yet all three models demonstrated hormesis, with similar quantitative features of the dose response. Nonetheless, despite this general predictive framework, it is important to acknowledge that few papers have made a strong effort to assess hormetic dose responses across a broad range of species or strains using similar experimental frameworks [46,47]. The studies cited above, however, have been supportive of the capacity to generalize the hormetic response.

ARE ALL AGENTS EXPECTED TO BE HORMETIC?

According to Stebbing [48,49], the key factor in the hormesis concept is not the chemical but, rather, the organism. In other words, the hormetic response is found in the organism's overcompensation to a disruption in homeostasis. If this is the case, then any agent that can disrupt homeostasis (i.e., cause toxicity) would be expected to induce a hormetic response to the damage induced. Calabrese et al. [46] recently explored this question using the U.S. National Cancer Institute Yeast Anti-Cancer Drug Screen database, which contains 2,189 chemical agents that were tested on 13 strains of yeast over five concentrations within a replicated study framework. That study established a priori entry criteria that included the demonstration of high concentration toxicity (i.e., decreased growth by at least 20%), an estimated benchmark dose or estimated toxicological threshold (e.g., 2.5, 5.0, or 10.0), and two or three concentrations below the benchmark dose for evaluation. Approximately 12,000 dose responses satisfied these entry criteria. These findings indicate that all 12,000 dose responses demonstrated evidence consistent with the hormetic dose response and supportive of the theoretical statements of Stebbing [48]. This is a new finding with potentially significant implications for chemical testing and risk assessment.

The Stebbing theory does not infer that all chemicals will be hormetic for all endpoints. It does, however, imply that biological systems respond in a hormetic manner to signals that indicate stress, toxicity, or disruptions in homeostasis.

ARE THERE CHEMICAL STRUCTURAL DETERMINANTS OF HORMESIS?

The above answer suggests that all chemicals have the capacity to induce a hormetic response in some experimental settings, but clear structural specificity exists in the induction of hormetic-like biphasic dose responses for specific endpoints and experimental conditions. This has been exploited in the pharmaceutical industry in search of biphasic dose responses that may lead to new drugs to reduce anxiety [50–52]. Many examples exist of agents that will differentially induce a U-shaped dose response to optimize a memory response using structure–activity relationship methods [53,54]. It therefore is necessary to place the above statement of Stebbing—namely, that all chemicals can induce hormesis—within a broader context. In these later cases, the U-shaped dose responses often appear to be acting via a specific receptor-mediated mechanism, providing an example of direct stimulation hormesis. The inhibition at the higher doses could result from a variety of mechanisms, including receptor desensitization, toxicity, or other factors.

ARE U-SHAPED DOSE RESPONSES FOR VITAMINS AND MINERALS EXAMPLES OF HORMESIS?

Various researchers have used the U-shaped dose responses for vitamins and minerals as examples of hormesis [55], but this is not recommended. The U-shaped dose responses in these instances represent organismal responses to nutrient deficits, optima, and excessive exposures. Hormesis studies usually have concerned agents that are not nutrients. In theory, however, if a nutrient were administered in excessive doses, causing a disruption in homeostasis, then it would be expected to induce a hormetic response. This would generate a different dose response than shown in the deficient optima/excessive dose response addressed in the above question (Fig. 11).

WHY DID THE CONCEPT OF HORMESIS BECOME ASSOCIATED WITH HOMEOPATHY?

The concept of hormesis became associated with homeopathy because Hugo Schulz thought that his findings that low doses of chemical disinfectants biphasically affected the metabolism of yeasts provided the scientific explanatory principle of homeopathy, an idea he actively promoted for nearly the

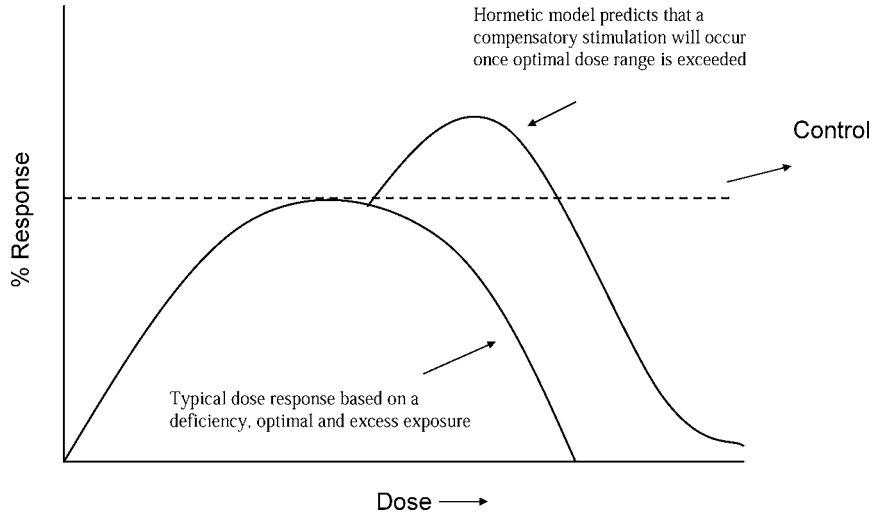


Fig. 11. Hypothetical hormetic dose–response relationship for a nutrient.

next 50 years. In addition, the field of homeopathy embraced the findings of Schulz, and thereby, the association was created. It is important, however, to understand how Schulz came to link his work with homeopathy.

According to his autobiography, Schulz became interested in how homeopathic medications may work [13]. During his first few years at the University of Greifswald in the early 1880s, Schulz became interested in the effect of a homeopathic remedy for the treatment of gastrointestinal enteritis. Schulz was convinced that the remedy was effective, but he wanted to understand why. In 1882, Robert Koch's laboratory identified the bacterial organism that was the cause of the disorder. Schulz exposed such cultured bacteria to the homeopathic preparation, expecting it to kill the microbes. To his surprise, the homeopathic treatment had no effect, even at progressively higher doses. Schulz concluded that the homeopathic treatment did not directly affect the harmful bacteria. Because he believed that the treatment was successful in a clinical setting, however, he developed the hypothesis that the drug enhanced the adaptive capacity of the body to fight off the infection rather than directly killing the bacteria. With this as his general hypothesis, he concluded, when he subsequently observed that low doses of chemical disinfectants stimulated the metabolism of yeasts at low doses while being harmful at higher doses [11,12], that this must be how homeopathic remedies work. He believed that these findings could be widely generalized and argued that his work provided the theoretical foundations of homeopathy.

IS HORMESIS MECHANISTICALLY ASSOCIATED WITH HOMEOPATHY?

Recently, it has been proposed that homeopathy has the potential to be evaluated within the context of postconditioning hormesis [29]. This conclusion is based on the research of van Wijk and Wiegant [36], who published an experimental model designed to place homeopathy within a rigorous and mechanistically oriented biomedical framework. Using a liver tumor cell line, these investigators set forth to create a model that would mimic how patients might be treated within a homeopathic context. In this case, liver cells initially were administered a low heat stress that was shown to induce a family of heat shock proteins. This preconditioning treatment offered partial protection against a more massive exposure to heat stress. The cells were then administered various metals and other agents/physical stressors that also have the capacity to induce heat shock proteins, but not at the low concentrations tested. This exposure to the low levels of metals and physical stress was after the massive heat administration. In this experimental setting, the below-threshold doses of these agents/physical stressors enhanced the induction of the heat shock proteins and reduced the toxicity and lethality of the massive heat treatment. These findings suggested that this homeopathic evaluation model would be suitable for study within the framework of postconditioning hormesis [29]. Whereas this research was undertaken during the 1990s and did show methodological promise along with encouraging findings, it has not been extended by other investigators. Nonetheless, this experimental framework was created by a group of highly experienced researchers in the area of heat shock proteins and could offer direction to future researchers. The lack of research suggests the existence of intellectual and cultural impediments that researchers encounter even if they attempt to test homeopathic hypotheses within a appropriate biomedical framework. It is hoped that eventually, the scientific questions raised by the work of van Wijk and colleagues [36] will attract others to build on their findings.

WHY DID THE CONCEPT OF HORMESIS FAIL TO BECOME ACCEPTED BY THE SCIENTIFIC COMMUNITY?

Several reasons explain why the hormetic dose–response concept was not accepted within the scientific community. A major reason was that Hugo Schulz made a profound strategic error when he aligned his new findings so strongly with homeopathy, setting it on an unfortunate and unnecessary collision course with the traditional medical establishment and its scientific leadership. Because of unfair characterization of his work, Schulz's findings became associated with extreme, high-dilutionist elements of the homeopathic medical practice.

Some of the most senior and accomplished pharmacologists of this era used their positions, power, and publications to ensure that the hormesis concept would not be widely accepted. Most notably was A.J. Clark, a leading pharmacologist in the United Kingdom during the 1920s until his death in 1941 and whose articles [56] and textbooks [57,58] excoriated Schulz, his theories, and homeopathy in a manner that made it difficult to separate them. Casting considerable influence on the field

for several decades via his textbooks, Clark played a significant role in suppressing the hormesis concept [42,59–61].

A key feature with intellectual control is that it can soon be expanded to institutional control. For example, Clark and his colleagues also became the cofounders of the British Pharmacology Society, influencing and directing research, advice to the government, as well as journal development and direction [42]. These activities also influenced the thinking of the next several generations of scientists. Of further importance was that the British Pharmacology Society had a significant impact of the development of pharmacology and other biomedical sciences throughout Europe and the United States.

Toxicology—especially human-oriented toxicology—emerged directly from pharmacology. In fact, the creators of the toxicology profession in the United States during the middle decades of the 20th century were principally pharmacologists transitioning into toxicologists. This perspective is further reinforced by the fact that the original journal of the U.S. Society of Toxicology (SOT), which was established first in 1960, was called *Toxicology and Applied Pharmacology*.

Another significant impediment for the hormetic concept was that when the first biostatistics model (i.e., probit) was applied to toxicology in the mid-1930s by Bliss [62], Gaddum, and Fisher (along with the assistance of Clark), it was designed to constrain all responses through the origin even if the data were J-shaped. This procedure became institutionalized and is still employed in risk assessment by the U.S. Environmental Protection Agency (U.S. EPA). During this time, toxicology became a high-dose/few-doses discipline, making it nearly incapable of providing the experimental framework to observe, assess, and study possible hormetic dose–response hypotheses. Thus, the hormesis concept was losing the battle for acceptance and credibility on many fronts: Medically, statistically, and academically; in professional societies, textbook content, research funding, and regulatory applications; and in the education and training of the next generation of biological/biomedical scientists.

To make matters even more difficult for the acceptance of hormesis, its evaluation requires considerable rigor with respect to study design, statistical power, and need for study replication. Hormesis, therefore, is not easily studied, being more expensive and time-consuming than traditional high-dose studies. Thus, hormesis became a scientific concept that was ridiculed and marginalized by accomplished and influential pharmacologists. In some cases, it was discounted by leading biostatisticians and eliminated from funding consideration by traditionally trained biomedical scientists in influential positions/roles. It may be difficult to accept that a legitimate scientific hypothesis could be purposely and successfully suppressed in the most open of countries, but this was the case with hormesis.

The rebirth of the hormesis concept came about almost entirely because of the extreme risk assessment policies of the U.S. EPA with respect to cancer endpoints. The development of acceptable risks in the one-in-a-million range over a normal human lifetime based on animal model studies that could never be validated became very economically burdensome in the early 1980s, and they remain so today. This stimulated affected parties to explore other means to challenge linearity at low-dose modeling. The obvious choice was to support the threshold dose–response model, both because there seemed to be much support for it and because little reason existed to think that carcinogens would not act via thresholds as well. In actual comparisons between threshold and linear models in specific dose–response studies, however, it became nearly impossible to distinguish the two types of dose responses given the limited number of doses in standard toxicity experiments. In such cases, the U.S. EPA would always default to the prediction with the greatest risk. Therefore, the analysis was quite clear: The linear model could not be challenged successfully by the threshold model. The alternative strategy was to explore the long-discredited hormesis model. This situation began to force scientists to take a new look at an old theory. The hormesis story involves far more than a challenge to linearity models used by regulatory agencies, but ironically, the conservative stance of the U.S. EPA on cancer risk assessment is what gave new life to the hormesis concept.

DID RESEARCHERS OBSERVE HORMESIS INDEPENDENT OF HOMEOPATHY IN THE EARLY 20TH CENTURY?

During the early 1900s, numerous reports of biphasic dose responses appeared in the literature by investigators researching the effects of various chemical agents on plants [63–68], bacteria [17,69–76], yeast [77], and fungi [78,79] (for review, see Calabrese and Baldwin [80]). Of particular note, these investigators presented their results in a typical scientific investigator fashion, not relating it to homeopathy or other medical treatment theory but, rather, simply as a new set of scientific findings.

Despite the occurrence of hormetic-like dose responses using various biological models by different leading researchers in the early decades of the 20th century, the concept of a low-dose stimulatory response was always hard to prove, requiring more resources, time, and need for replication. Nonetheless, despite its lack of capacity to become a central concept in toxicology, researchers continued reporting hormetic-like effects in the literature, yet it was not until later in the 20th century that serious efforts were made to assimilate this information and to evaluate claims of hormesis in a substantive and objective manner [1,48,49,81–84].

DO ALL ENDPOINTS DISPLAY HORMESIS?

Based on the above answer, it is believed that all organisms may display hormesis in response to a disruption in homeostasis. Furthermore, it is known that hundreds of endpoints have been shown to be stimulated in a hormetic manner [10], depending on the biological model and the chemical tested. Thus, it appears from a practical perspective that most, if not all, endpoints have the capacity to display hormesis.

A broad range of endpoints has demonstrated hormetic dose responses. What endpoints are measured are directly related to the goals of the research team. Endpoints that typically show hormesis, however, are those that represent integrative biological responses, some of which are related to resource allocation. Such endpoints could include growth, viability, cognition, longevity, and coordinated immune responses, such as cell migration to affected areas. It is not clear which quantitative features of the responses interact in such a manner as to affect the reaction of the molecular vector that is the integrated biological response called the hormetic dose–response relationship [85].

A critical factor affecting endpoint selection nonetheless is the biological model studied. In the case of hormesis, its proper study requires numerous doses, repeated measures, and adequate statistical power and replication. To minimize costs, these factors have led to a large proportion of the early findings

being obtained with inexpensive and more manageable biological models. These have included the use of plants, bacteria, yeasts, and fungi [80,84]. In the case of plants, it has been common to assess possible hormetic effects with endpoints such as overall growth, fruit yield, disease resistance, and other endpoints of agricultural application. In the microbial area, the principal focus for hormetic response evaluation initially concerned colony proliferation. Over the past two decades, a major shift to the use of cell culture and, more recently, to the application of high-throughput studies has provided efficient and inexpensive means to assess hormetic dose responses. The cell culture research has been important for a broad range of biological models, from microbial to a broad spectrum of human cell lines. Recent rodent toxicity studies demonstrate hormesis for a broad range of endpoints, including disease incidence, tumor formation, and reproductive endpoints, such as fecundity. During the past several decades in the pharmacological area, hormesis also has been demonstrated for a broad range of performance endpoints, such as memory, anxiety reduction, pain modulation, seizure modulation, and reduction in the onset of symptoms from diseases such as Alzheimer's Parkinson's, and others.

Some disease endpoints cannot be assessed directly within a hormesis evaluative framework if the model has a very low background disease incidence. For example, if the incidence of liver disease is less than 1% in the controls, it will be practically impossible to evaluate whether a low dose of a hormetically acting agent would reduce the disease incidence further. In this case, liver disease may be an effective endpoint for a chronic study in which disease incidence in the control group increases over time. This strategy would not likely be effective in a short-term study with young animals. This situation is not unlike that which occurs with the testing of cancer incidence in animal models. From a historical perspective, it was an advantage for regulatory agencies to adopt the use of animal models with a low background incidence of cancer. This permitted the use of a smaller number of animals to detect significant increases in tumor incidence compared with studies using biological models with higher background tumor incidence. Selecting animals with lower tumor incidence, however, also tended to prevent one from being able to detect hormesis. Thus, the capacity to detect a significant increase in tumor incidence with a small sample size and to detect a hormetic effect for the same endpoint have been in conflict with each other. This issue needs to be addressed explicitly as new strategies are employed in hazard assessment for the detection of responses across the broad dose–response continuum rather than following current protocol, which ignores potential hormetic effects.

HOW DOES HORMESIS RELATE TO THE MIXTURE TOXICOLOGY?

Hormesis principally deals with biological performance—that is, the response of biological systems below the toxic threshold. Above the toxic threshold, the shape of the dose response is similar for the threshold and hormetic dose–response models. A number of studies have explored chemical interactions within a hormetic framework. In most cases, chemical interactions such as synergy and potentiation have been reported. Responses were within the modest increase limits of the hormetic stimulatory response. In other words, whether or not synergy existed, the maximum stimulation was 30 to 60% greater than the control response range. To achieve such modest increases, the dosage of a drug/chemical can be markedly reduced if synergy or potentiation occurs within a hormetic context. The hormetic type of synergy therefore has far less to do with the magnitude of the response than with the amount of drug/chemical to achieve this hormetic maximum. This has been reported with respect to memory [86–88], epileptic seizure threshold [89], and plant growth [90], among other endpoints. Therefore, the concept of synergy at the hormetic end of the dose–response relationship is a different type of biological process than synergy at the upper end of the dose response for toxic endpoints. Hormetic synergy means achieving the maximum potential (i.e., 30–60% above controls) with a diminished combined dosage. The hormetic synergy concept is one that deals with biological performance, such as cognition, exercise, anxiety modulation, hair growth, and other goals. It is not the traditional type of toxicological synergy, in which the output is principally on the magnitude of the toxic response. This new type of biological synergy has profound implications for the pharmaceutical industry that is focused on enhancing performance outcomes.

DO HORMETIC RESPONSES OCCUR IN BOTH ACUTE AND CHRONIC STUDIES?

Hormesis has been reported to occur in experimental studies, independent of study duration and life span of the species. This is seen in studies where the responses are of short-term occurrence, such as a 12-h change in proliferation rate of yeast [46], or with the enhancement of life span in rodents [91,92] as measured over several years. An agent could, however, induce hormesis in the first part of an experiment but toxicity in a longer-term exposure if the agent (e.g., cadmium, which has a long biological half-life) were to accumulate and transition to a toxic concentration in the target organ. Thus, the occurrence of hormesis is highly dependent on the pharmacokinetics of the agent in the biological model. The impact of pharmacokinetics has even been reported during the course of a single administration [93]. For example, morphine, a well-known analgesic, acts as a hypergesic (i.e., increasing the magnitude of pain responses) at very low doses. In studies with rats, it has been shown that soon after morphine administration, the pain threshold decreases; later, as the dose to the target organ increases, the pain threshold increases only to decrease again as the dose to the target organ decreases, all occurring in a matter of hours [93].

IS THERE A RELATIONSHIP BETWEEN THE ADAPTIVE RESPONSE IN TOXICOLOGY AND HORMESIS?

The adaptive response in toxicology was given this name in 1978 by Schendel et al. [94], following the 1977 paper of Samson and Cairns [95], which indicated that a low dose of a mutagen protected against the mutagenic effects of a more massive exposure to the same agent. The low-dose exposure induced an error-free DNA repair process that was effective over a defined dose range. From this beginning, the concept of adaptive response was confirmed, expanded, and generalized beyond bacteria and mutagens to be inclusive of a very wide range of biological models, endpoints, and chemicals. A key feature relates to the exposure sequence, with a previous low dose inducing a complex array of adaptive responses to protect the system against a subsequent and more massive exposure to the same or a related agent. It also was recognized that the duration over which the protection lasted was of a limited nature—that is, from several days to approximately 10

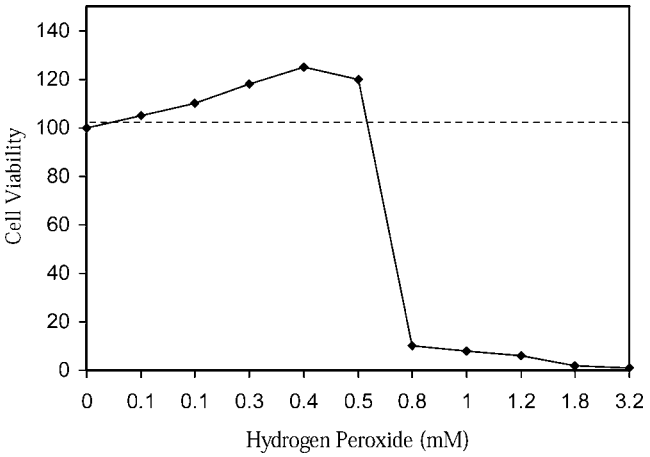


Fig. 12. Effect of hydrogen peroxide on cell viability of *Saccharomyces cerevisciae* strain R253 [96].

to 14 d at most. Nearly forgotten in this assessment was that the adapting or conditioning dose had an optimal range. This dose–response range displayed the quantitative features of the hormetic dose response (Fig. 12) [96]. Thus, the adaptive response phenomenon represents a specific type of hormesis that Calabrese et al. [29] refer to as the preconditioning hormesis.

IS IT HEALTHY TO BE CONTINUOUSLY STRESSED IN A HORMETIC SENSE?

One can be stressed daily via caloric restriction, intermittent food ingestion, exercise, and other ways and, thereby, induce hormetic mechanisms that prevent disease processes and enhance health outcomes [97]. It appears that to optimize health, biological systems need to be routinely stressed, with the quantitative and temporal features of that stress response conforming to the hormetic dose response. This question also may pose the challenge of assessing whether agents can turn on downstream hormetic mechanisms while bypassing toxicity. In fact, this would be a long-term research goal with very significant biopharmaceutical and public health implications.

DOES HORMESIS OCCUR INDEPENDENT OF AGE?

Most research in rodent models concerning hormesis has been performed with relatively young adults, but published research with very young animals also has demonstrated the occurrence of hormesis. There have been reports in which hormetic responses were age dependent, occurring only after adulthood was reached [98]. However, whereas it appears that hormesis can occur in different age groups, this is not an area that has been systematically assessed.

DOES HORMESIS OCCUR IN BOTH SEXES?

Considerable data demonstrate that hormetic effects occur in both sexes [10]. The quantitative features of the dose response also are similar between males and females, including the maximum stimulatory response, width of the stimulation, and relationship of the maximum stimulation to the toxic threshold.

DOES HORMESIS OCCUR IN HEALTHY AND DISEASED STATES?

This area has not been addressed in a detailed fashion. It is not possible to offer any data-based generalizations regarding this topic.

DOES HORMESIS OCCUR IN INDIVIDUALS FROM LEAST TO MOST SUSCEPTIBLE?

This issue was addressed by Calabrese and Baldwin [98], who reported that hormesis and its quantitative features occurred largely independent of susceptibility to toxic agents. The more susceptible subjects simply displayed their hormetic dose responses downshifted to the left. In such cases, the susceptibility to the agent in question was not related to the hormesis response. In some cases, the hormetic response was absent in the susceptible subgroups, and this may have been a factor in the observed increased susceptibility.

WHAT IS THE MECHANISM OF HORMESIS?

No single mechanism accounts for the general occurrence of hormetic dose responses. This would not be expected, because hormesis likely occurs in most, if not all, plant, microbial, and animal species; in essentially all tissues and organs; across a broad spectrum of endpoints; and independent of chemical and physical stressor agents. The constraining of hormetic responses at a maximum of 30 to 60% greater than controls, however, regardless of model, endpoint, and agent, suggests a common and highly conserved strategy that remains to be elucidated.

HAS HORMESIS EVER BEEN MECHANISTICALLY EXPLAINED?

A large number of specific hormetic dose responses have been explained mechanistically in some level of detail, often to the level of receptor and, in some instances, to steps farther downstream [4,85]. For the past 30 years, mechanistic explanations have been offered for biphasic dose–response relationships [24]. The general biological strategy to achieve a biphasic dose response has been to use two receptor subtypes that bind to the same agonist, one leading to a stimulatory or inhibitory pathway. In this case, the agonist would have differential affinity for both receptor subtypes, along with differential receptor capacity (i.e., number of receptors). In general, the agonist may bind one receptor subtype with far greater efficiency than it does the other receptor, thus activating its pathway at low doses. If the receptor with less binding affinity has greater capacity than the other receptor, however, then at higher doses, it would become dominant and induce the inhibitory response. If this relationship were plotted, it would appear as a hormetic-like biphasic dose response. This type of scheme has been demonstrated repeatedly in various receptor families. It is believed to be very generalizable, and it has been applied to numerous biological agents/systems, such as prostaglandins [99], estrogens [100], androgens [101], adrenergics [102], adenosine [103], 5-hydroxytryptamine [104], dopamine [105], opiates [106], amyloid β-peptide [107], peptides [108], apoptosis [109], and cell migration/chemotaxia [110].

IS A SINGLE MECHANISM REQUIRED FOR A BIPHASIC DOSE RESPONSE TO BE CALLED HORMESIS?

No requirement exists for a single mechanism to account for the hormetic biphasic dose–response relationship. In fact, over the past several decades in the field of pharmacology, biphasic dose responses typically have been explained by two or more interacting mechanisms, as noted in the answer in the previous section.

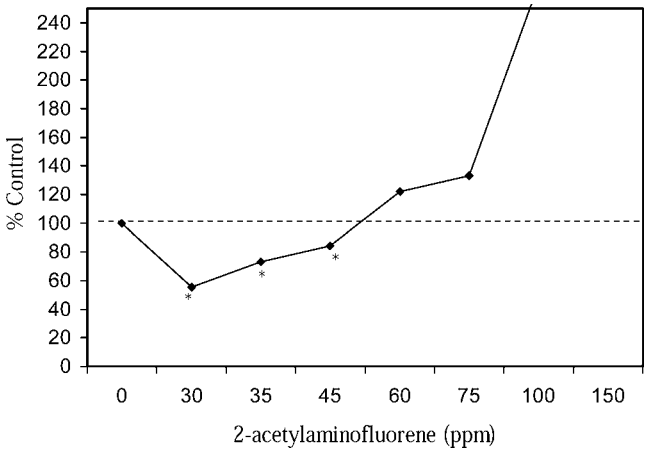


Fig. 13. Incidence of bladder tumor adjusted for time in ED01 megamouse study [1,121].

HOW MANY CHEMICALS HAVE HORMETIC MECHANISMS DESCRIBED BASED ON DATA?

As noted above, numerous agents have had their hormetic dose response assessed in detailed mechanism-oriented studies. Many of these findings have been identified and summarized in reviews [2,3].

WHY DO MANY TOXICOLOGISTS BELIEVE NO MECHANISTIC EXPLANATION FOR HORMESIS EXISTS?

Even though recognition of the hormetic dose response is growing in the field of toxicology, it is commonly stated, even among supporters of hormesis, that little mechanistic understanding regarding this phenomenon exists. In fact, a recent book by Rodricks [111] makes precisely this point. Strong evidence is available to dispute this conclusion. Rodricks is far from alone in this belief, however, because toxicologically based mechanistic explanations for the hormetic dose response have been of limited value. On this point, Rodricks and others would be correct. Toxicological mechanism research has not been designed to account for dose-dependent changes in the dose–response relationship. This has been a strong focus in the pharmacological sciences, however, and therein are provided numerous examples of mechanistic explanations that account for the occurrence of hormetic dose responses. In essence, the field of toxicology has been far behind the field of pharmacology when it comes to providing mechanisms that account for hormetic biphasic dose–response relationships.

DO GENOTOXIC CARCINOGENS ACT HORMETICALLY?

This has long been a contentious issue. The U.S. government attempted to answer the question regarding the nature of the dose response in the low-dose zone for the model genotoxic carcinogen, 2-acetylaminofluorene (2-AAF), in the largest-ever rodent cancer bioassay during the late 1970s, in which more than 24,000 animals were tested [112–114]. This was such a highly significant event that the SOT created an independent group of 14 experts to separately analyze the findings, with the SOT devoting nearly an entire issue of one of its journals to this expert group's assessment [91]. In this 1981 report, it was determined that 2-AAF induced cancer in the bladder and liver at high doses, as was expected. In the case of the bladder cancer, the results indicated a clear and significant J-shaped dose–response relationship (Fig. 13), a finding that was emphasized by the authors of the SOT report.

The authors of the SOT report never mentioned the term hormesis, but they were insistent that the risk of bladder cancer was decreased in the below-threshold zone, the dose response being clearly consistent with the hormetic dose response. The findings of the J-shaped dose response occurred in each of the six rooms housing the animals during the study, thereby providing a type of built-in experimental replication. With respect to the liver cancer, the number of doses was insufficient to resolve the nature of the dose response.

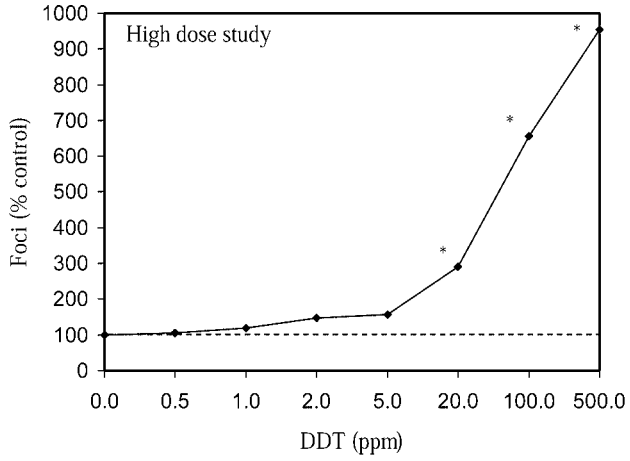
The 2-AAF study, now referred as the megamouse study or the ED01 study, was resource intensive and may never be undertaken again with rodents. In this one-of-a-kind study, however, using a model carcinogen and involving enormous previous planning to ensure adequate testing, the hormetic dose response for the bladder cancer response was a definitive finding. Despite the strong conclusion of the SOT expert panel, it is interesting to note that several years later, when the SOT distributed a slide set on toxicology for teachers, the shape of the dose response for carcinogens was shown to be linear—a conclusion that was clearly in conflict with the findings of its own panel of 14 experts regarding the bladder cancer endpoint.

DO EPIGENETIC CARCINOGENS ACT HORMETICALLY?

This question has been investigated in considerable detail by Japanese investigators using a variety of epigenetic carcinogens [115–119]. In general, these investigators reported that when studied over a very broad dosage range, the responses at high dosages increase the occurrence of tumors and/or liver foci formation, which is an excellent predictive marker for liver tumor development. As the dosage was progressively lowered, however, the opposite response occurred, and the risk of developing either liver cancer or foci significantly dipped below that of the control group (Fig. 14). These studies were very strongly designed with respect to concerns about the number of doses, proper dose spacing, and statistical power. In addition, considerable attention was directed toward assessing the underlying mechanisms that could account for the enhanced cancer risk at high dosages and the reduced cancer risks below threshold dosages in the hormetic dose–response range. The quantitative features of the dose responses in the series of papers published by the Japanese investigators generally were consistent with those reported within the hormesis database [115,116,119,120]. In contrast to the lack of mention by the SOT expert panel in 1981 [121] of the term hormesis for the responses of 2-AAF in terms of bladder cancer incidence, the Japanese investigators viewed their findings as being manifestations of hormetic dose response. Based on this extensive set of experiments and publications, it can be reasonably concluded that some epigenetic carcinogens act in a hormetic fashion in rigorously designed rodent carcinogen bioassays.

DO TUMOR PROMOTERS ACT HORMETICALLY?

Some tumor promoters or their metabolites that act via the inhibition of cell-to-cell communication have been reported to enhance such activity at lower doses, showing the biphasic dose–response features of hormesis [122–125]. These data suggest that promoters may have the potential to reduce tumor promotion at lower doses while enhancing the process of carcinogenesis at higher doses. For example, the benzene metabolite hydroquinone biphasically affected cell-to-cell communication in IARG1 cells [123]. Hormetic-like enhancement of cell-to-cell communication also was reported for menedione



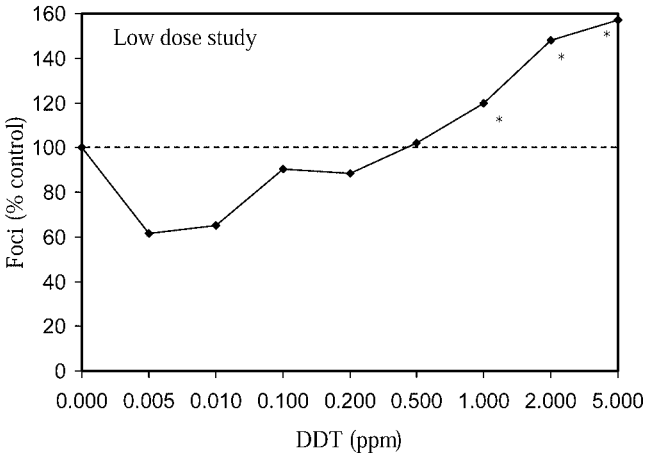


Fig. 14. Effect of DDT on number of glutathione-*S*-transferase P-positive foci in F344 rat livers in bioassays assessing different but slightly overlapping doses of carcinogen. Note that as the dose decreases, the J-shaped dose–response curve becomes evident. Also, note the difference in scale between the two graphs [1,119]. Asterisks denote statistical significance.

and H_2O_2 (Fig. 15) [123] as well as for retinoic acid (Fig. 16) [124].

WHY DID REGULATORY AGENCIES INITIALLY ASSUME THAT CHEMICALS AND RADIATION ACT VIA A THRESHOLD DOSE–RESPONSE MODEL?

The threshold dose response became established in the 1930s based on earlier supportive data [126,127] and following development of the probit model constraining of responses to approach control data in an asymptotic manner using the maximum likelihood estimate [42]. This model was then quickly applied to numerous biological fields via numerous publications by Bliss [62,128–136]. The rapid acceptance of the threshold model came at the expense of the Arndt-Schulz law (i.e., hormesis) alternative. The threshold model had the authority of the leading pharmacologists in Europe, as lead by A.J. Clark, and the support of the leading biostatisticians, such as R.A. Fisher. The hormesis model had been discredited by Clark through his linking it with the high-dilutionist elements of homeopathy. The path became clear in such circumstances for the regulatory scientists in the United States to reject hormesis and accept the threshold model, especially because most of these decision makers were graduates of traditional medical schools trained in pharmacology. Within a very short period of time, the perspectives embraced by Clark and his colleagues became institutionalized.

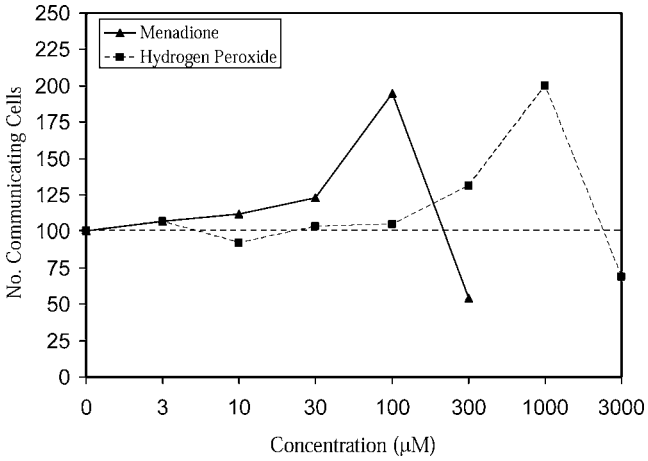


Fig. 15. Gap junction intercellular communication (GJIC) levels in BPNi cells exposed for 1 h [123].

WHEN AND WHY DID REGULATORY AGENCIES CONCLUDE THAT CARCINOGENS MAY ACT VIA A LINEAR FASHION AT LOW DOSE?

Regulatory agencies concerned with the health effects of radiation were influenced by the research of Muller during the late 1920s and 1930s [137,138] that suggested radiation may induce mutations in a linear fashion. This led to an erosion of confidence in the threshold dose–response model that had been used to assess radiation-induced injury. By the mid-1950s, national and international radiation advisory committees decided that radiation cancer risks should be seen as stochastic events [139], leading to rejection of the threshold model for cancer and adoption of a linear-at-low-dose model prediction strategy for assessing cancer risks [140–142]. With respect to chemical assessment, nothing definitive occurred until the U.S. Congress passed the Safe Drinking Water Act in 1974, requiring that the U.S. EPA authorize the National Academy of Sciences (NAS) to create a Safe Drinking Water Committee to advise on how to assess toxic substances in drinking water. In 1977, the NAS published its long-awaited book *Drinking*

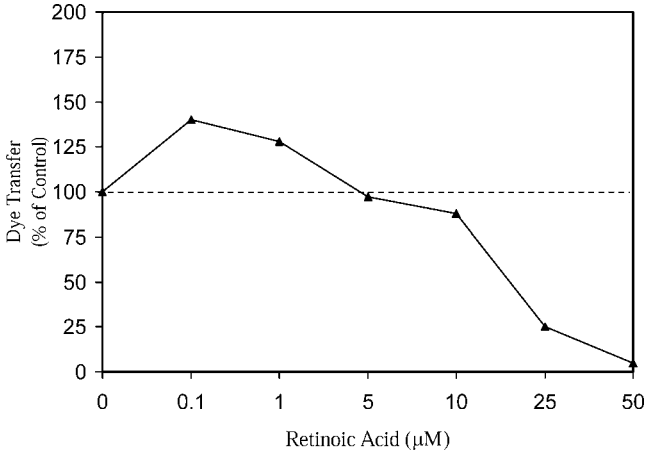


Fig. 16. Effects of retinoic acid on dye transfer in IAR 203 cells [125].

Water and Health, which contained the recommendation to accept linearity at low doses as the means of estimating risks from carcinogens. The NAS committee did no original thinking during this process but, rather, accepted the strategy of nearly 20 years earlier for assessing radiation-induced cancer risks. The U.S. EPA quickly followed the recommendations of the NAS and started down the path of applying linearity at low doses to a large number of chemicals that had been shown to be carcinogenic in animal models using the few-doses/high-doses testing scheme. It is important to note that the discovery of the adaptive response to chemical mutagens by Samson and Cairns [95] was submitted to *Nature* in December 1976 and accepted in March 1977. The book *Drinking Water and Health* did not cite this paper; however, one could only imagine what the course of cancer risk assessment might have been if the NAS Safe Drinking Water Committee had been made aware of these findings in time to affect its linear-at-low-dose recommendations, which were to be challenged by the adaptive response model.

ARE STUDY DESIGNS USED BY REGULATORY AGENCIES CAPABLE OF ASSESSING HORMESIS?

Study designs that do not include an adequate number of doses below the toxicological no-observed-adverse-effect level are incapable of observing hormetic dose responses. No reasonable likelihood exists, therefore, that current regulatory requirements for hazard assessment will detect possible hormetic dose responses by design, but only by accident. In addition to study design, it is necessary to take into account the background disease incidence of the control group. If the control group has a very low disease incidence for endpoints of interest, then there will be little capacity to observe possible hormetic effects. In general, biological models selected by regulatory agencies have a low susceptibility to infectious diseases as well as a low background incidence for the disease of interest. The desire for the low background incidence of chronic disease was a reasonable goal, because it would reduce the sample sizes needed to demonstrate a treatment-related significant response. It also, however, would make it impossible to study hormetic dose–response relationships, as noted above.

SHOULD HORMESIS BE THE DEFAULT MODEL IN RISK ASSESSMENT?

The basis for how a default risk assessment model should be selected needs careful consideration with broad scientific discussion. Numerous issues need to be considered, including, among others, the biological plausibility of the models; the capacity of the models to be validated; the strategy for hazard assessment, including study design and statistical power considerations; the relationship of biological mechanism to biostatistical model selection; and the capacity to extrapolate model findings to general population data. In the case of the U.S. EPA, influential working groups [143] have indicated that the purpose of a risk assessment is to provide estimates of exposure that predict toxicologically based dose–response relationships. Their process assesses the occurrence of toxic effects and their underlying modes of action. While acknowledging that adaptive responses can occur, they state that the adaptive response area is outside their focus. Therefore, the U.S. EPA does not assess the entire dose–response continuum, only that portion that starts at the point of demonstrable increases in adverse effects.

The U.S. EPA position is remarkable in that it acknowledges not only the possibility and, indeed, the likelihood of adaptive responses but also its clear intention not to consider such responses in their evaluation. Knowledge regarding the shape of the dose response across the entire dose–response continuum can have important public health implications. For example, if a J-shaped dose response occurred in which risk was reduced by approximately 50% below background cancer incidence, the U.S. EPA would not consider whether and how this could be used for society's benefit. It simply would not approach this, even in a theoretical sense.

The U.S. EPA also continues to use a default model for carcinogens in risk assessment that cannot be validated. That is, the low level of estimated risks (e.g., $<1/1{,}000$) simply cannot be realistically assessed in an experimental framework. The decision to use a practically unvalidated model by regulatory agencies is based on the prevailing public health protectionist philosophy that less is always better whenever exposure to chemical and physical stressor agents/toxic substances are concerned. It should be pointed out that hormetic dose responses can be readily tested, assessed for their accuracy, and validated or rejected. Acceptance of linear-at-low-dose modeling, however, has provided regulators with the option of using hazard assessment protocols with very few high doses, simply because it is very easy to model linear relationships across two to four doses that are in the above-threshold response domain.

An important aspect of default model selection is whether this model offers accurate predictions in the low-dose zone. To date, two major head-to-head comparisons of the threshold and hormesis models have been conducted [46,144,145]. Not only did the hormesis model far outperform the threshold model, but more importantly, the threshold dose–response model performed extremely poorly. The tests were not designed for hormesis to do well and its challenger to do poorly. It simply appears that the threshold model does not describe biological reality well for doses below the toxicological threshold, whereas the hormesis model does a far better job. The question therefore is not so much why the U.S. EPA does not accept the hormesis model but, rather, why it stands behind models that clearly perform poorly in predicting responses in the low-dose exposure zone.

MODE OF ACTION VERSUS MECHANISM OF ACTION FOR CARCINOGEN RISK ASSESSMENT AND HORMESIS

In 1996, the U.S. EPA established the requirement/goal for having a mode of action to guide risk assessment model selection [146]. The mode-of-action concept is far from a complete or even substantial understanding of a mechanism of action. For example, an agent may be positive in a genotoxic study but also carcinogenic. The U.S. EPA can simply determine the mode of action to be via its mutagenic activities, yet with little insight regarding its specific mechanism. The identification of mode of action was deemed to be an important decision point, because it could affect whether a threshold or a linear-at-low-dose model could be employed in the risk assessment process. What the mode of action is determined to be is critically important, because it classifies the agent into a risk assessment process box of either threshold or linear.

In the case of hormesis, there often has been a demand to know what the mechanism of action is before hormesis can be accepted and used in the risk assessment process. It is ironic that for hormesis, a demand exists for the mechanism of action

to be known whereas the U.S. EPA only requires a mode of action to justify its selection of either a threshold or a linear-at-low-dose model. In the case of hormesis, numerous and well-known modes of action exist. Would a mode of action for hormesis be satisfied if it were receptor mediated? Would it be necessary to identify the specific receptors that are activated and inhibited? Would it be necessary to identify mechanisms farther downstream before the hormesis mode of action would be satisfied? If the U.S. EPA can use mutagenicity as a mode of action, then it would seem that a receptor-mediated mode of action would be equally general. With respect to hormesis, the quantitative features of the dose response are the same regardless of the mode of action or even with a theoretical mechanism of action. This suggests that the U.S. EPA requirement of a mode of action for model selection for hormesis has no theoretical foundation.

WHEN WAS THE HORMESIS CONCEPT FIRST USED IN REGULATORY PROCESSES?

The findings that low levels of arsenic and lead not only would not inhibit but would actually enhance plant growth was presented in a major regulatory hearing in California (USA) in 1912 during an evaluation of a major smelter facility [147]. The research was authorized by the state regulatory process and was conducted at the University of California (Berkeley, CA, USA) by a well-regarded researcher (i.e., Charles Lipman) who was to become Dean at that institution [147–152].

ARE BACKGROUND EXPOSURES TO IONIZING RADIATION ABOVE, WITHIN, OR BELOW THE HORMETIC ZONE?

Luckey [153] frequently has stated that the environment in which humans now reside is far below the hormesis zone for ionizing radiation, because there has been substantial decay of radionuclides over billions of years. In the case of other agents, this has not been addressed. Luckey cited studies with 10 different organisms in which a reduction in ionizing radiation below normal background level led to adverse effects on various parameters, such as growth and viability. Whether this also is the case with humans remains to be assessed.

WHY IS IT DIFFICULT TO PROVE HORMESIS?

The assessment of hormesis demands that toxicologists employ stronger study designs, along with greater statistical power, than they commonly have done. It also requires a more careful set of preliminary studies to initially estimate the no-observed-effect level/no-observed-adverse-effect level so that doses can be spaced properly both above and below the estimated toxic threshold. Because the maximum response is likely to be modest, careful consideration must be given to sample size to employ adequate statistical power. This also requires that the investigators have a very good understanding of the background variation within the control group. Depending on the endpoints to be measured, it also may be critical to incorporate repeat sampling or measurements over time. This would provide the opportunity to identify initial toxicity and possible compensatory responses. If hormetic effects are observed, they will be of a modest magnitude and, usually, will require adequate replication of the findings. The above research scheme is not really difficult, but it requires more resources and time to confidently define the nature of the dose response in the low-dose zone.

IS ABSOLUTE PROOF OF HORMESIS POSSIBLE?

There does not appear to be a means to prove, in an absolute sense, that hormesis has occurred in a specific case. Firm, statistically based conclusions, however, can be drawn that hormesis has occurred if the studies are well designed, with adequate numbers of doses, proper dose spacing, and sufficient statistical power and replication of findings. If the mechanism for the low-dose stimulation is receptor mediated, then it may be possible to further strengthen the case by the use of synthetic agonists and antagonists to deconstruct and reconstruct the dose response.

WHY IS THE MAXIMUM STIMULATION MODEST?

The reason that the maximum hormetic stimulation is consistently modest in magnitude has never been an objective of detailed evaluation. Within the past few years, however, the suggestion has been raised as to whether the term ceiling effect in pharmacology for the maximum response of a pharmacological dose response may represent the maximum stimulation as seen in the hormesis database [41]. A careful consideration of how the concept of ceiling effect has been used in pharmacology suggested that these two diverse sets of observations may be addressing the same concept—that is, the maximum of the hormetic dose response.

Whereas the ceiling effect concept now is widely used, no attempt has been made to assess why its magnitude is modest. As noted above, the magnitude of the stimulation is similar across cell types, agonists, and biological models, independent of the proportion of receptors. It appears that the maximum stimulatory response may reflect a response potential that is constrained by the plasticity of the biological system, which appears to be highly generalizable based on the thousands of dose responses within the hormesis database.

DOES HORMESIS SUGGEST THAT BIOLOGICAL SYSTEMS IMPROVE PERFORMANCE BY ONLY 30 TO 60%?

Ten years ago, an answer of yes would have seemed to be an obviously incorrect response. Now, having viewed many thousands of hormetic dose responses [10], the answer would appear to be a very firm yes. The implications of these observations are profound, because they place limits on what pharmaceutical companies expect to achieve at the maximum response with a drug treatment. It also will inform the strategy of biostatisticians designing studies, by knowing in advance that a possible treatment effect will not exceed approximately 30 to 60% compared with the controls. From a more philosophical and futurist perspective, it would seem to be possible that the biological limitations of the ceiling effect might be able to be engineered around, genetically altered, or biologically manipulated to achieve several- to many-fold increases in performance rather than the low percentage increases built into the hormetic perspective. It is not clear, however, what this might mean biologically if such highly conserved limitations were bypassed. This, therefore, is an important question to be considered.

HOW DID THE DOSE–RESPONSE CONCEPT DEVELOP WITHIN PHARMACOLOGY?

The concept of dose response in pharmacology had a number of independent formulations during the early decades of the 20th century. The credit, however, goes to Clark [57] and his efforts in the area of quantitative pharmacology to place

the dose response on solid theoretical, biomathematical, and population-based foundations. His textbooks, which were updated and republished over a 40-year period, profoundly influenced two generations of pharmacologists and toxicologists in the middle decades of the 20th century. Clark's work clearly established the primacy of the threshold dose–response model and facilitated the incorporation of the probit model into bioassays in numerous biological disciplines. The textbooks of Clark also severely criticized the concept of hormesis and the work of Hugo Schulz, significantly affecting the impact of this dose–response concept throughout the remainder of the 20th century.

HOW DID THE HORMESIS CONCEPT DEVELOP WITHIN PHARMACOLOGY?

Whereas the term hormesis has not been widely used in the field of pharmacology, the area of biphasic dose responses became recognized in the late 1970s based on a large number of independent reports in the pharmacological literature. These observations were assessed by Szabadi [24] and integrated into a mechanistically based dose–response theory that involved the activities of opposing receptor subtypes that differentially bound to the same agonist. This theoretical framework was repeatedly verified and expanded over the next three decades [154–156]. The relationship of this mechanistically derived model is that the quantitative features of the dose response are similar to those seen within the hormesis database.

IS THE POSSIBILITY OF CHANGING BIOLOGICAL SET POINTS AFFECTED BY THE CEILING EFFECT?

A biological set point in dose–response terms may be thought of as the ceiling effect. Now, if this ceiling effect could be considered as the new control group or new baseline, could the ceiling or set point be raised again? If this were the case, it would create a wide range of biomedical possibilities. For example, if a drug could increase memory in patients with Alzheimer's disease by 30%, could this then be built on and added to, thereby improving performance markedly rather than marginally. Many other possibilities could be raised. Some investigators have attempted to alter set points, especially with respect to drug addiction [157]. In such cases, the set points have been increased, but very modestly and still within the constraints of the quantitative features of the hormetic dose response. Thus, at present, it does not appear that changing biological set points has been—or is likely to be—easily achieved.

ARE ANY PHARMACEUTICAL AGENTS BASED ON HORMESIS?

Numerous agents currently employed by the pharmaceutical industry act via hormetic mechanisms, displaying hormetic dose responses. In the historical development of these drugs, however, none was said to have acted via a hormetic dose response. It has been demonstrated that numerous antianxiety drugs act via a hormetic-like biphasic dose–response relationship, yet this has not been presented within a context of hormesis until recently [41]. This also is the case for seizure drugs, pain medication, and numerous other clinical pharmacological applications. Drugs commonly act via a hormetic dose response; however, the term has yet to be used in this area.

HOW DOES HORMESIS RELATE TO THE CONCEPT OF TOLERANCE?

Tolerance represents an adaptation of biological systems following prolonged exposures to agents of concern. In the process of tolerance development, a dose that induced biological effects at low doses eventually cannot induce that effect under the same experimental conditions. A higher dose is required to induce the same effect in the tolerant subject. The relationship of tolerance to hormesis has been extensively explored in the case of ethanol consumption in multiple mouse strains. In this case, ethanol exposures have been reliably shown to induce a low-dose stimulation of locomotion and a higher-dose inhibition. As a general rule, however, a chronic tolerance develops only to the higher-dose inhibitory effects of ethanol, not to the low-dose stimulatory effects [158–161]. Consequently, at least as far as the effects of ethanol in multiple mouse models are concerned, the hormetic response was independent of the development of tolerance. It remains to be assessed, however, whether this specific relationship of hormesis to tolerance can be extended to other models, endpoints, and agents.

ENDOCRINE DISRUPTORS AND HORMESIS: WHAT IS THE RELATIONSHIP?

Estrogenic endocrine-disrupting chemicals (i.e., xenoestrogens) often induce an inverted U-shaped dose response, based on a low-dose stimulation. The inverted U-shaped dose response of endocrine-disrupting agents displays the same quantitative features as do those described for hormetic dose responses. Because hormesis is defined as a dose–response phenomenon characterized by a low-dose stimulation and a high-dose inhibition, xenoestrogens inducing such biphasic dose responses clearly would be considered examples of hormesis. Calabrese and Baldwin [30] indicated that hormetic dose responses may occur as an overcompensation to a disruption in homeostasis or as a direct stimulatory response. The quantitative features of the overcompensation or direct stimulatory pathways are indistinguishable. They also are difficult to differentiate in a practical sense, because most toxicological experiments do not conduct dose–time–response examinations, the time component being critical for assessing the overcompensation phenomenon. It also was emphasized that the definition of hormesis should be decoupled from whether the response would be considered beneficial, harmful, or unknown. Thus, it is believed that xenoestrogens can be effectively evaluated within a hormetic context. This has been disputed by Weltje et al. [162], who argue xenoestrogens most likely would induce effects that would not be considered beneficial and that do not seem to display overcompensation responses. It should be noted that xenoestrogens could act in a potentially harmful manner on one tissue while being beneficial within another. For example, in the case of bisphenol A, the possibility exists that it could increase prostate size at a low dose, a response that might be considered nonbeneficial, but that it may enhance neuroprotection [163] in a similar biphasic dose–response manner, also via an estrogen receptor–related mechanism. The key point is that hormetic-like biphasic dose responses predominate within numerous biological systems, with the quantitative features being remarkable similar and their biological and biomedical interpretations often being challenging.

Numerous agents that display hormetic-like biphasic dose responses that are not xenoestrogens can have a potentially adverse effect as a result of the low-dose stimulation, thereby

making the xenoestrogens not unique in this regard. For example, some agents have been reported to increase anxiety at low doses while decreasing anxiety at higher doses [164]. Calabrese [2] reported that a sizable proportion of antitumor agents stimulate the proliferation of human tumor cells at low doses while killing these cells as the dose increases. Some agents that act biphasically on the immune system cause harmful effects (e.g., lupus, tuberculin sensitivity, enhancement of viral infectivity and autoantibody formation) in the low-dose stimulatory zone [3]. Certain cardiac glycosides, such as ouabain, enhance the proliferation of smooth muscle cells comprising the prostate gland, with the possibility of obstructing urine flow and of doing so in a manner consistent with the hormetic dose response [165]. The statin drug family [166] is known for its capacity to enhance capillary formation and, thereby, possibly increase cancer risks in affected tissues. These are examples that illustrate that endocrine disruptors are not unique in their capacity to induce potentially harmful effects within the low-dose stimulatory domain of the hormetic-like biphasic dose response. This is a commonly observed consequence, each with unique mechanisms but all following the some dose–response pattern, the same quantitative features of the dose response, and all being constrained by the plasticity limits imposed on their respective systems. By providing a broadly encompassing intellectual framework for biphasic dose–response evaluation, including those of endocrine disruption, it is expected that the hormetic dose response provides an effective vehicle for concept integration and interdisciplinary terminological consistency without restricting the application of this information for use in the biomedical sciences and in risk assessment practices.

WHY IS HORMESIS IMPORTANT TO TOXICOLOGY AND TOXICOLOGISTS?

Hormesis is important to toxicology, because the central pillar of this field is the dose–response relationship. Data over the past decade have indicated that the field of toxicology made a crucial error regarding its most fundamental and central feature—that is, the dose response. The field of toxicology made the assumption that the threshold model was reliable because thresholds were readily apparent. The threshold dose–response model also predicted that responses to doses below the toxic threshold would vary randomly on either side of the control group's value. Even though below-threshold doses define most exposures, the threshold model response assumption was not assessed formally until 2001. At that time, Calabrese and Baldwin [145] first established that the threshold dose–response model poorly predicted responses below the threshold whereas the hormesis model did so with high efficiency, findings that have since been refined and extended [46,144]. The failure to adequately understand the nature of the dose response in the low-dose zone by the toxicology and regulatory communities has led to hazard assessment protocols and risk assessment practices that are based on a faulty understanding of the dose–response relationship in the critical low-dose range. The fact that current default dose–response models have been shown repeatedly to poorly predict responses in the low-dose zone continues to be an error of considerable scientific, public health, and economic significance.

Why did this happen? As recounted above, the issues are complex and interwoven, but the answer may be distilled to a few leading contenders: The long and hostile battle between traditional medicine and homeopathy; the linkage of Schulz's findings to homeopathy; the need for traditional medicine to defeat its opponent at all costs, even if the opponent's data are solid; the establishment of political, institutional, and financial control over the development of the field, including its funding and research directions, thereby further marginalizing its opposition; establishing statistical procedures that deny the existence of the opposing theory and employ some of the most prestigious and accomplished scientists in the process; insidiously censoring scientific ideas and information from subsequent generations of scientists in free and open societies; and convincing the public that this scientific system is acting in their best interests. On top of all this, the opposing theory—namely, that of hormesis—also was very difficult to prove, requiring far more resources and time, always appearing to be a marginal response, and being easily confused with background variation if not studied rigorously. In the end, it was easy to suppress hormesis.

Toxicology has been a discipline that is supposed to inform decision makers about the nature of the dose response across the entire dose–response continuum. It did the easy stuff well—that is, identifying and describing toxicity at high doses. Once that easy problem was solved, toxicology struggled and failed with the issue of our time—namely, the nature of the dose response in the low-dose zone. The hormesis concept is applying the proverbial smelling salts to the field of toxicology and risk assessment. It appears that it may be getting a response from the fallen giants, but at this stage, it is not certain that the field of toxicology will be able to right itself, establish more accurate toxicological bearings, and thereby, better serve the interests of society.

Acknowledgement—Sponsored by the Air Force Office of Scientific Research, Air Force Material Command, under grant FA9550-07-1-0248. 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 the official policies or endorsement, either expressed or implied, of the Air Force Office of Scientific Research or the U.S. Government.

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APPENDIX 1

Hormetic principles

Low/modest stress induces pro-survival responses.
The quantitative features of the hormetic dose response are similar across species and individuals and independent of differential susceptibility and agent potency.
The magnitude of the stimulatory response is constrained by and defines the plasticity of the biological system.
Hormetic responses occur at multiple levels of biological organization, such as the cellular, organ, individual, and population levels.
Downstream processes integrate responses from multiple independent stressor agents/excitatory stimuli to yield an integrated dose response (i.e., molecular vector) reflecting the hormetic dose response.
Hormetic responses reflect both a general response to environmentally induced stress/damage as well as some elements of chemical structure specificity for end point induction.

APPENDIX 2

Major hormetic dose–response observations

Most commonly observed dose–response relationship.
Distinctive quantitative features, making it a unique biphasic dose–response relationship.
Most unique feature is the modest magnitude of the stimulatory response, usually less than twice the control values.
The low-dose stimulation can occur via a direct stimulation or via an overcompensation to a disruption of homeostasis.
Hormetic dose responses may be seen as an adaptive response that ensures tissue repair in an efficient manner and protects against damage from subsequent and more massive exposures.
Hormetic dose responses are highly generalizable, being independent of biological model, end point measured, and chemical class.
Numerous specific mechanisms have been reported to account for hormetic dose responses.

APPENDIX 3

Implication of hormesis for toxicology/risk assessment and clinical practices/pharmaceutical companies

Toxicology/risk assessment

- Changes strategy for hazard assessment, altering animal model, end point selection, and study design, including number of doses, dose range, and number of subjects per dose.
- Alters biostatistical modeling to predict estimates of response below control background disease incidence.
- Differentiates dose optima (i.e., benefits) for normal- and high-risk segments of the population.
- Creates evaluative framework to assess benefits or harm below traditional toxicological threshold.
- Creates new framework for quantitatively altering the magnitude of uncertainty factors in the risk assessment process.

Clinical practices/pharmaceutical companies

- Drug performance expectation will be constrained 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., tumor cell proliferation).
- Modification of biological set points will be constrained by the quantitative features of the hormetic dose response.
- Clinical trials need to recognize interindividual variation in the hormetic dose response.
- Clinical trials need to be designed to take into account the quantitative features of the hormetic dose response.

APPENDIX 4

Biomedical/clinical applications of immune-related hormetic effects

Agent	Clinically favorable effect
Whole-body radiographs	Reduce tumor metastasis
Radiographs	Enhanced antibody titer
Tucaresol	Human immunodeficiency virus treatment
Numerous bryostatins	Antileukemic agents
Opioids	Tumor reductions
Cytokine modulation	Acute respiratory disease
N-acetylcysteine	Treatment of respiratory disease
Isoprinosine	Treatment of respiratory disease
Cystamine	Liver/kidney disease conditions
Osbeckia extract	Liver disease conditions
Methimozole	Graves' disease
Fungicide	Decreased fish disease
Estradiol	Bacterial/viral disease reduction
Corticosteroid	Bacterial/viral disease reduction
Indomethacin	Bacterial/viral disease reduction
Antirheumatic drugs	Bacterial/viral disease reduction
Alcohol	Bacterial/viral disease reductionq
Coumarin	Bacterial/viral disease reduction and antitumor effects
Levemisol	Bacterial/viral disease reduction
Chlorpromazine	Bacterial/viral disease reduction
Opioids	Bacterial/viral disease reduction
Allicin	Tumor reduction
Retinoic acid	Treating leukemia patients
Resveratrol	Antitumor effects
Agent	Clinically unfavorable effect
Procainamide	Lupus
Antirheumatoid agents	Tuberclin sensitivity
Cocaine	Enhance viral infectivity
Hydrazine	Autoantibody formation