RulemakingComments Resource

From: Edward <edwardc@schoolph.umass.edu>
Sent: Wednesday, June 24, 2015 7:41 AM
To: RulemakingComments Resource
Subject: [External_Sender] Linear No-Threshold Model and Standards for Protection Against Radiation; Notice of Docketing and Request for Comment
Attachments: taapmargintomainstream.pdf; Horrmetic Mechanisms-1.pdf; SETAC-1.pdf; SETAC-ETC-2011-1.pdf

Dear NRC:

I am writing in support of the proposed change to an hormetic dose response model by the NRC for carcinogen regulation. I am currently attaching
four published papers that provide a scientific basis for this proposal.

Sincerely,

Ed Calabrese, Ph.D.
Umass/Amherst
Professor of Toxicology

RulemakingComments Resource

From: Edward <edwardc@schoolph.umass.edu>
Sent: Wednesday, June 24, 2015 8:09 AM
To: RulemakingComments Resource
Subject: [External_Sender] Linear No-Threshold Model and Standards for Protection Against Radiation; Notice of Docketing and Request for Comment
Attachments: Hormesis and LNT-1.pdf; Hormesis Data Base-2011.pdf; Hormesis and Medicine-BJCP-pdf.pdf; HET-Hormesis-15 years.pdf

Dear NRC:

I am writing in support of the proposed change to an hormetic dose response model by the NRC for carcinogen regulation. I am currently attaching four published papers that provide a scientific basis for this proposal.

Sincerely,

Ed Calabrese, Ph.D.
Umass/Amherst
Professor of Toxicology

RulemakingComments Resource

From: Edward <edwardc@schoolph.umass.edu>
Sent: Wednesday, June 24, 2015 10:49 AM
To: RulemakingComments Resource
Subject: Re: Question on rulemaking comment(s) submitted to the NRC

Dear Herald:

I sent two emails because I did not think that there was enough memory on my end to attach all eight. There are eight different supportive articles.
I hope this is clarifying.

Sincerely,

Ed Calabrese
On 6/24/2015 10:40 AM, RulemakingComments Resource wrote:

> Dr. Calabrese,
>
> Attached are copies of the 2 emails we received from you last night. The language in the body of the email is the same but the 4 attachments have different titles. Do you consider them separate comments because the attachments are different, or are they duplicates with retitled attachments? If duplicates, we would process only one of the comments, likely the one that came in later, under the assumption you made some correction to it.
>
> Would you please clarify your intention at your earliest opportunity so that we can add your comment(s) to the docket?
>
> Thank you.
>
> Herald Speiser
>
> *******************************
> ***Herald M. Speiser***
> ***Rulemakings and Adjudications Staff***
> ***Office of the Secretary***
> ***U. S. Nuclear Regulatory Commission***
> ***(301) 415-1675***
> *******************************

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Review

Hormesis: from marginalization to mainstream
A case for hormesis as the default dose-response model in risk assessment

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Abstract

The paper provides an account of how the hormetic dose response has emerged in recent years as a serious dose-response model in toxicology and risk assessment after decades of extreme marginalization. In addition to providing the toxicological basis of this dose-response revival, the paper reexamines the concept of a default dose model in toxicology and risk assessment and makes the argument that the hormetic model satisfies criteria (e.g., generalizability, frequency, application to risk assessment endpoints, false positive/negative potential, requirements for hazard assessment, reliability of estimating risks, capacity for validation of risk estimates, public health implications of risk estimates) for such a default model better than its chief competitors, the threshold and linear at low dose models. The selection of the hormetic model as the default model in risk assessment for noncarcinogens and specifically for carcinogens would have a profound impact on the practice of risk assessment and its societal implications.

Keywords: Hormesis; U-shaped; J-shaped; Dose response; Biphasic; Carcinogen; Adaptive response

Introduction

Within the past 2 years, there has been a considerable interest in the topic of hormesis, a dose-response phenomenon characterized by a low-dose stimulation and a high-dose inhibition (Calabrese and Baldwin, 2002). This phenomenon is typically seen as an inverted U-shaped or a J-shaped dose–response relationship depending on the endpoint measured (Fig. 1). This interest has become manifest not only in the popular media (e.g., *Discover*—December, 2002; *Fortune*—June, 2003; *Scientific American*—October, 2003, *London Times*—October, 2003; *Forbes*—December 2003; *Boston Globe*—December, 2003; *Wall Street Journal*—December 2003), but also in the highly visible scientific publications such as *Nature* (February 13, 2003) and *Science* (October 17, 2003), and the more focused publications within the fields of toxicology and pharmacology (e.g., *Toxicological Sciences*, *Human and Experimental Toxicology*, *Toxicology Letters*, *Annual Reviews of Pharmacology and Toxicology*, *European Journal of Molecular Biology*, etc.).

The question of why the topic of hormesis has achieved such widespread attention quite recently is of interest because the concept of biphasic dose–response relationships emerged well over a century ago in the 1880s (Schulz, 1887) although the term hormesis was coined about 60 years ago by researchers at the University of Idaho (Southam and Ehrlich, 1943) (see reviews by Calabrese and Baldwin, 2000a, 2000b, 2000c, 2000d, 2000e on the history of the dose response in chemical toxicology and radiation biology). In fact, ever since its initial discovery by Schulz in the mid-1880s, the concept of hormesis has had a difficult time getting established within the legitimate scientific hierarchy in large part as a result of its early and close association with the medical practice of homeopathy (Bellavite et al., 1997, 1997a), as well as the long-standing interest of the toxicology community and regulatory agencies in high-dose effects and the modest nature of the low-dose stimulatory hormetic response, thereby making it more difficult to establish and replicate than high-dose toxicity effects.

The current principal motivation that provided the incentive to reassess the hormetic hypothesis has been the conservative approach for regulating exposure to carcinogens by agencies such as EPA. The adoption of a "linear at low dose"

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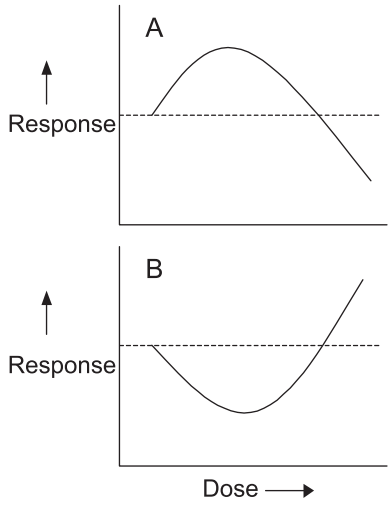

Fig. 1. (A) The most common form of the hormetic dose–response curve depicting low-dose stimulatory and high-dose inhibitory responses, the β- or inverted U-shaped curve. Endpoints displaying this curve include growth, fecundity, and longevity. (B) The hormetic dose–response curve depicting low-dose reduction and high-dose enhancement of adverse effects. Endpoints displaying this curve include carcinogenesis, mutagenesis, and disease incidence.

assumption, that is, the linear no threshold (LNT) model, to estimate cancer risks has had such a large impact on the field of risk assessment and the costs to implement exposure standards and clean up actions that U.S. industry began to explore the hormetic hypothesis in the mid-1980s in an inquisitive but not a fully engaged serious manner. The critical early elements motivating this reevaluation can be more or less traced to the influence of a 1981 book on *Ionizing Radiation and Hormesis* by Thomas Luckey and a conference inspired by the Luckey book on *Radiation Hormesis* in 1985 held in Oakland, CA, under the leadership of the late Dr. Leonard Sagan of the Electric Power Research Institute (EPRI). Spin-off activities from this conference eventually lead to a systematic and detailed evaluation of the hormesis hypothesis via activities of BELLE (Biological Effects of Low Level Exposures—http://www.belleonline.com).

Over the course of this literature assessment activity, nearly 6000 dose–response relationships consistent with the hormesis hypothesis have been obtained from the peer-reviewed literature which satisfied a priori evaluative criteria (i.e., strength of study design, magnitude of stimulation, statistical significance, and reproducibility of findings) to varying degrees. As a result of this activity, a relational retrieval database was created; several articles describing quantitative features of the hormetic response and its potential for generalizability were also published (Calabrese and Baldwin, 1997, 1997a, 1998; Calabrese et al., 1999).

Quantitative features of the hormetic dose response

An assessment of the hormesis database has revealed important information concerning the nature of the dose response in the low-dose zone, that is, at doses below the traditional No Observed Adverse Effects Level (NOAEL) that has been often regarded as a quasi-toxicological threshold. The assessment of the quantitative features of the dose response revealed that the hormetic stimulation is generally quite modest. About 80% of the maximum stimulatory responses have been less than twice the control value with the maximum stimulation being approximately 30–60% greater than the control response in most cases. We have come to hypothesize that large stimulatory responses that exceed something on the order of 4- to 5-fold most likely represent phenomena that may be different than hormesis.

The hormetic response is contiguous with the traditional dose–response relationship in the toxicological literature, becoming manifested immediately below the toxicological NOAEL. The stimulatory range of hormesis effects appears to be more variable than the amplitude of the hormetic response. This stimulatory response typically extends over a dose range of 20-fold or less immediately below the NOAEL. In fact, the majority of stimulatory responses (approximately 60%) have been less than 10-fold. However, about 8% of the stimulatory ranges have exceeded a dose range of 100, although nearly half of these (4%) are greater than 1000-fold. Among the endpoints (i.e., growth, metabolic endpoints, reproduction, and survival) with at least 250 dose responses in the database, immune responses stood out as displaying by far the greatest proportion (23%) of stimulatory responses exceeding 1000-fold, whereas others were $\leq 4\%$.

The modest stimulatory responses present significant challenges in studying hormesis because it is difficult to differentiate hormetic responses from those of background variability. As a result, it has been common to reject the hormesis hypothesis due to concerns of background variability along with a modest response assessed within the context of inadequate study designs (e.g., inadequate number of doses, improper dose spacing below the NOAEL, lack of statistical power, and no consideration for multiple temporal samplings) and lack of replication of the experimental findings. It is precisely for these reasons why our criteria for judging the likelihood of hormetic responses impose rigorous requirements for study design, statistical significance, and replication of findings.

The continuity of the hormetic dose response with the traditional toxicological dose response is perhaps its critical feature, providing a predictable component of the dose–response relationship, starting immediately below the NOAEL. The predictability of the qualitative and quantitative features of the dose response has important theoretical and practical implications for the field of risk assessment that may consider both beneficial and harmful effects as well as for clinical medicine as physicians consider placing a patient either in or out of the hormetic zone. The variability in range of the hormesis zone has implications for study design, including the total number of doses and dose spacing employed. The modest stimulatory response also suggests some measure of biological significance for

numerous applications. That is, a 30–60% increase may be important in some areas but not necessarily others.

The use of the above evaluative criteria has been important in establishing the scientific foundations of the hormetic dose response. The net result has transformed the hormesis hypothesis from its past uncertain, and, at times, shunned status, to one of considerably enhanced credibility. That is, hormetic dose responses satisfying a priori objective criteria are now recognized as having been published by hundreds of independent research groups over the past century and in a broad variety of journals in the toxicological and biomedical subdisciplines passing hundreds of peer review processes. Based on an assessment of these findings, it is no longer reasonable or credible to deny the biological reality of the hormesis phenomenon.

The hormesis database has also established that the hormetic response occurs in a broad variety of plant and animal models, with no apparent restriction to endpoint measured as well as chemical class and physical agent tested. This perspective is viewed as especially significant because it establishes a broad-based biological and chemical agent generalizibility of the hormesis concept.

Frequency of hormesis in the toxicological literature

Despite the growing recognition of the hormetic phenomenon, the issue of the significance of hormesis is a different and more sophisticated question. Although there are numerous examples of hormesis in the toxicological and pharmacological literature and that this phenomenon is broadly seen within a wide range of biological models, endpoints, and agents, the question of the frequency of hormesis in the literature is not answered by these observations. That is, although it was possible to conclude that hormesis existed and generalizable, the present data did not reveal whether it occurred in 1%, 50%, or 80% of possible cases, an issue of concern, especially for regulatory agencies, such as EPA. If hormesis occurs in less than 1% of properly designed studies, then it could be recognized as real but dealt with as an exception to the rule. If, however, it occurred, for example, in greater than 20% of properly designed studies, then it may be considered as part of the rule.

The frequency question was addressed with the creation of an entirely new (i.e., Hormesis Database II—Frequency) database which included a priori entry criteria as well as a priori evaluative criteria. With both numerator and denominator, the frequency of hormesis in the toxicological literature could be estimated. Based on this second hormesis database, the frequency of hormesis in the toxicological literature was estimated at approximately 40% (Calabrese and Baldwin, 2001). This represented a significant advance because it provided a legitimate estimate of the frequency of hormesis within the toxicological literature. Because the criteria are rigorous, the actual frequency was probably higher than this value.

This "frequency" hormesis database was further exploited to assess which dose-response model is more frequent in the toxicological literature, that is, the traditional threshold model, which has been nearly universally accepted, or the hormesis model. The threshold model assumes that responses of doses below the NOAEL should not be significantly affected by treatment doses. That is, below NOAEL doses are generally accepted as being below the effect "threshold." If this were the case, then the responses to such doses should randomly vary on either side of the control value. However, when the nearly 1800 responses to below NOAEL doses from nearly 700 dose–response relationships in the Hormesis Database II–Frequency were evaluated, the majority displayed values greater than the controls, a collective response highly consistent with the hormetic hypothesis (Calabrese and Baldwin, 2003). Thus, not only was an estimate of the frequency of hormesis in the toxicological literature obtained but that under head-to-head comparison conditions the hormetic model clearly outperformed the threshold model. This observation leads to the conclusion that the hormetic dose-response model is more frequent than the long-accepted universal standard, the threshold model, in appropriately designed toxicological studies.

This surprising finding leads to the question of how could the toxicological community have missed the basic reality of the hormetic dose response in favor of the threshold model because the dose–response relationship is so basic to toxicology. Although there are numerous possible factors affecting the historical rejection of the hormetic model (Calabrese and Baldwin, 2000a, 2000b, 2000c, 2000d, 2000e), the principal scientific factors affecting its rejection by the toxicological community lie in the fact that the scientific discipline of toxicology has long been focused on assessing high doses, with the goal of trying to define the NOAEL and LOAEL in traditional hazard assessment protocols. This high-dose toxicological world was also embedded into experimental protocols that used very few doses, that is, most studies had a control group and only two or three doses. With such a limited number of doses and with the focus on generating NOAELs and LOAELs, it is not unexpected that 98% of the nearly 21 000 toxicological studies assessed in the creation of the second hormesis database were unable to satisfy the study's data entry criteria due to inadequate study designs (Calabrese and Baldwin, 2001). Another reason why the toxicological community failed to adequately recognize the hormesis hypothesis is that the NOAEL often displays a low level of toxicity although the response is not statistically significantly different than the control. This is also the case for some doses immediately below the NOAEL dose (Calabrese and Baldwin, 2003).

This combination of factors (e.g., focus on high doses, inadequate study designs, residual toxicity in the NOAEL and adjacent lower dosages, etc.) has contributed to the major error of the toxicological community over the past

century that the most basic dose-response model is the threshold model and not the hormetic model. This continuing 'mistake' has been truly of historic proportions affecting the most basic toxicological concepts, teaching curricula from elementary to graduate school, and risk assessment methods that have provided the scientific basis of regulatory actions and societal risk communication.

Mechanistic foundations

The next challenge that the hormetic dose-response model hypothesis had to confront was the issue of mechanism. In modern toxicology, there has been a strong tendency to worship at the altar of mechanism. This strong focus on mechanism, indeed, has critical advantages including the capacity to generate better biologically based hypotheses, which are much more likely to yield greater explanatory and predictive insights. Based on discussions with leading toxicologists, it became evident that in order for hormesis to achieve a central status, it was necessary to adequately address the issue of mechanism. Although the current primary focus concerned toxicologically based hormetic responses, very little toxicological mechanism research dealt specifically with accounting for changes or switches in the dose–response relationship. In contrast, there has been considerable research in the pharmacological literature on dose–concentration response relationships in which switches in the dose response had been explicitly assessed and with considerable success. Our research has revealed several dozen receptor systems (e.g., adenosine, adrenergic, bradykinin, cholecystokinin, corticosterone, dopamine, endothelin, epidermal growth factor, estrogen, 5-HT, human chorionic gonadotropin, nitric oxide, prolactin, numerous opioids, prostaglandin, somatostatin, testosterone, etc.) in which hormetic-like dose–response relationships had been reliably reported and where mechanisms have been determined to at least the level of the receptor and often to considerably greater levels of biological complexity (Calabrese and Baldwin, 2001a, 2003a, 2003b). In such research, it was common for investigators to deconstruct and reconstruct their dose responses via the use of various synthetic agonists and antagonists specific to the above receptor systems that confirmed the underlying mechanistic basis of the hormetic dose–response relationship.

Hormetic-like biphasic dose–response relationships acting via receptor-based mechanisms were typically explained from having two receptor subtypes with markedly different agonist affinities which lead to either a stimulatory or inhibitory pathway. At low concentrations, the receptor subtype with the greatest affinity would display its response. The receptor subtype with the lower affinity for the agonist would also have the higher capacity, that is, more receptors. At higher concentrations, the receptor subtype with the lower affinity and higher capacity would become more dominant. As a result of the combined presence of both receptor subtypes, it is possible to both demonstrate and explain in mechanistic terms hormetic-like biphasic dose–response relationships. Although the above explanation describes a common and general form by which biphasic dose–response relationships take place, there are a variety of permutations with different levels of complexity and interactions by which such responses occur.

One of the basic findings that has emerged from the mechanism-based research is that there is no single hormetic mechanism. In fact, there are numerous ways in which biological systems can manifest hormetic-like biphasic dose–response relationships. What is seen among the array of hormetic mechanisms is a general commonality in the quantitative features of the hormetic dose response. This modest amplitude of the stimulatory response suggests that hormetic responses operate within a mechanism framework that is designed to conserve resources. Such a limited, yet efficient, use of resources is also consistent with the hypothesis that hormesis often appears as modest overcompensation to a disruption in homeostasis and may represent a broad biological strategy although specific mechanisms unique for each system are simply biological tactics. The overcompensation hypothesis has a long history, being first reported as early as 1897 by Townsend and then later supported by Branham (1929), Hoektoen-Spelling (1920), Smith (1935), Taliaferro and Taliaferro (1951), and Warren (1945). More recently, Calabrese (2001) greatly expanded the list of examples of dose-response studies supportive of the overcompensation hormesis hypothesis. An assessment of these experiments indicates the need for a large number of doses and also a possible repeat measure or temporal component to assess the overcompensation dose-response phenomenon. Multiple dose/temporal experiments which provide the best theoretical understanding of hormesis as an overcompensation to a disruption on homeostasis have been assessed in only a small proportion (i.e., approximately 500) of the overall database.

The cause of variability in the width (e.g., approximately 5 to >1000-fold) of the stimulatory response of hormetic dose–response relationships is not well studied. However, preliminary assessment of our data suggests that the width of the stimulatory response may be accounted for, at least in part, by heterogeneity of the study population and the relative proportions of various subgroups that comprise the study subpopulations.

Because the hormetic dose-response model is highly generalizable according to biological model, endpoint measured and agent tested and to be far more common than the threshold dose response in head-to-head comparisons using a priori entry and evaluative criteria, and can be validated in experimental settings, a critical feature lacking in LNT model, the issue may be raised as to whether the hormetic model could replace the threshold and linear models as defaults for estimating acceptable exposures of noncarcinogens and carcinogens, respectively.

What is a default dose-response model

A default model is typically viewed as a dose-response model selected by various regulatory agencies for routine use in deriving acceptable exposures to toxic substances either of a noncarcinogenic or carcinogenic nature in the absence of convincing evidence to the contrary. In the United States, the EPA, which has broad-based regulatory authority for exposure to toxic substances, utilizes the threshold dose-response model for the assessment of exposures to noncarcinogenic agents and the linear at low doses multistage model to estimate risk from exposure to carcinogenic agents. There is a social value component in the decision-making process with a tendency of default models to estimate higher risks at lower doses, thereby leading to more conservative exposure standards than competitive models, that is, an erring on the side of safety. Nonetheless, acceptance of default models assumes that they would display some combination of superior overall theoretical foundation and experimental or empirical data to support its selection. The next section assesses the basis of the selection of current default dose-response models, that is, the threshold and linear at low dose model approaches for noncarcinogens and carcinogens, respectively.

Acceptance of the threshold dose-response model

The underpinnings of the threshold dose-response model are historically derived from the concept of the tolerance dose (see Kathren, 1996, for a review of this issue). In the initial decades following the discovery of radiation, it became generally accepted that a certain specific dosage of radiation can be sustained by various tissues without an adverse effect. This perspective lead to the concept of the tolerance dose, the level of radiation to which a person could be continuously exposed without demonstrable harm. The tolerance dose idea provided the basis for the first statement of a dose limit in the field of radiation. More specifically, the tolerance dose was based on a fraction of the erythema dose, that is, the dose required to produce a perceptible reddening of the skin (Mutscheller, 1925). According to Kathren (1996), the tolerance dose was clearly a threshold which was consistent with "the idea of recovery (or repair) from any subclinical acute effects with denial of the possibility of long term low level effects". Various international groups, such as the International X-Ray and Radium Protection Committee (1928), the forerunner of the International Commission of Radiological Protection (ICRP), and the U.S. Advisory Committee on X-Ray and Radium Protection (1929), the predecessor of the National Council on Radiation Protection and Measurements (NCRP), utilized the tolerance dose (i.e., threshold dose-response model concept) as the underlying basis of their exposure standards from the mid-1920s to the mid-1940s (Kathren, 1996). The tolerance dose, which was built upon the threshold dose-response model, was, therefore, a well-established concept that was fully integrated into international health decision-making actions. Of particular importance to the acceptance of the tolerance concept/threshold dose-response model was that it satisfied the requirement to protect workers, patients, and the general public from harm and that such conclusions were consistent with their observational experiences.

The threshold dose response in chemical toxicology has its origin in both community health standards such as with drinking water standards for agents such as lead that can be traced to at least as early as 1849 in England (Gueneau de Mussy, 1849, as cited in Wanklyn and Chapman, 1884), but principally with early attempts to protect worker health to a growing number of agents during the early and middle decades of the 20th century (Calabrese, 1978; Cook, 1945). Pharmacology and drug safety were also supportive of the threshold model, with it being endorsed within leading texts of the 1930s such as the highly influential *Handbook of Pharmacology* by A.J. Clark. Regardless of their different histories, the radiation health, chemical toxicology, and drug pharmacology domains relatively independently honed in on the threshold dose-response model to guide governmental and national/international advisory groups to protect human health.

The issue of carcinogen risk assessment did not emerge until later in both the chemical and radiation domains. The principal conceptual impetus that lead to the development of the linear dose-response model was the striking findings of Muller in 1927 which indicated that mutation rate in irradiated fruit flies appeared to proceed without a threshold and that the mutagenic effect of radiation is cumulative over a lifetime. These findings earned Muller the Nobel Prize nearly 20 years later (1946). These earlier intellectual seeds planted by the Muller research generated interest in the application of the linear non-threshold model for somatic genetic effects. The shift from the tolerance dose model to the linear non-threshold model was more of a revolution in thinking than gradual evolution once the thought galvanized interest. In fact, in 1954, the NCRP published its recommendations of permissible dose from external sources of radiation (NCRP, 1954) with the British following suit in 1955. These reports replaced the tolerance concept/threshold model with the idea of a maximum permissible dose (MPD) which suggested the notion of acceptable, rather than no risk. Thus, the linear model, which almost 30 years before had been born in the fruit fly research of Muller dealing with mutagenicity, was transformed into practical reality. By 1958, the UN incorporated such thinking into its radiation health framework along with the U.S. Federal Radiation Council in 1959 (see Kathren, 1996). These views were further reinforced in U.S. congressional hearings in 1959. Thus, within a 5-year period, a dose-response revolution leading to acceptance of

the linear no-threshold (LNT) model had been achieved at highly influential levels.

By the early 1970s, the U.S. National Academy of Sciences (BEIR I) offered cancer risk assessment estimates for radiation based on linear extrapolation, implying that there was no threshold in the low-dose zone. Paralleling these efforts with radioactivity, the NAS (1977) Safe Drinking Water Committee (SDWC), under a charge from the 1974 Safe Drinking Water Act, recommended the use of linear at low dose modeling for chemical carcinogens, a practice that has been employed ever since. The NAS (1977) essentially adopted the views of NAS BEIR I Committee (1972) against the existence of thresholds. They then made the extension from radiation to chemical carcinogenesis by stating that because ''many carcinogenic agents act like radiation in producing mutations, chromosomal aberrations, and cell killing, we see this as an additional argument against the likelihood of thresholds in the dose–response curve of these agents''. These conclusions were viewed as consistent with studies in mammals where the induction of various genetic alterations and tumors appeared linear with low-LET radiation up to about 50–100 rad (Barendsen, 1975; Brown, 1976). These perspectives were then integrated into a population-based framework in which the assumed biological reality of human heterogeneity in response to toxic substances presided, leading to difficulty in establishing a single threshold for a large and biologically diverse population. These views became easily reconciled and merged with theoretical quantitative theories of carcinogenesis in the early 1950s such as those from Iversen and Arley (1950) in which single normal cells were proposed to have some probability of being transformed to cancer cells with the rate of transformation being a linear function of the amount of carcinogen. This conceptual framework became the basis of the one-hit model of carcinogenesis. Later refinements in such modeling were offered by Armitage and Doll (1954, 1961) and later by Crump et al. (1976) and would lead to the linear at low dose default model of estimating cancer risks.

In contrast to the threshold dose-response model in which the responses seemed to coincide with human experience and therefore readily believable, the LNT model for carcinogens inevitably requires extensive extrapolation from high to low dose. That is, the findings are typically extrapolated from an observable response of 10–50% as seen in animal model bioassays to risks of one in a million or so in the human population. In such activities, it is not uncommon to require extrapolation over four to six orders of magnitude of dose.

In contrast to the generally accepted threshold dose-response model, there is no convincing evidence that the LNT model accurately predicts cancer risks at low doses and that even its theoretical foundation is not convincing especially in light of current molecular understandings of adaptive mechanisms even in subgroups at increased risk at low dosages. Furthermore, the LNT model is not able to be adequately tested in chronic bioassays even below the very high risk of $<1/100$ due to the limitations in resources. Moreover, the use of over 24 000 mice in the famous ED01 study could not resolve risks below 1 in 100, hence the name ED01 (Staffa and Mehlman, 1980). Consequently, even if risks were to be observed as linear down to a risk of 1/100 or somewhat less, it may not remain so if the dosage were further decreased. This leads to the logical conclusion that it would not be possible to prove which model was more likely correct by experimental studies. Given this situation, we are left with the current status, that is, historical strengths of the threshold model govern how noncarcinogens are assessed, although a conservative protectionist public health philosophy as seen via use of a linear at low dose model governs the cancer risk assessment domain.

Criteria in the selection of a default dose-response model

Although prior articulation of what should constitute the criteria of a default dose-response model in risk assessment is generally quite limited (see Calabrese and Baldwin, 1998), the above historical recapitulation suggests that the threshold and linear default models have been selected without systematic evaluation. Nonetheless, it would appear that selection of a default risk assessment model should address the following eight concerns.

Generalizability according to biological model, endpoints measured and range of chemical classes and physical agents

The capacity for generalizability is probably the most significant consideration for a default model because it provides the broadest and most reliable framework to base predictions on. The findings for hormesis have been evaluated in detail and are based on thousands of dose–response relationships that have satisfied evaluation criteria as noted above.

Frequency of model responses in the toxicological literature

The frequency issue has been difficult to resolve because individual experiments in toxicology have such few doses that it is generally not possible to differentiate threshold from linear models especially in the area of carcinogenicity where low-dose extrapolation modeling predominates. The data from the same experiments are often consistent with both linear and threshold models. In contrast, research findings on the frequency of hormetic dose responses in the toxicological literature (Calabrese and Baldwin, 2001, 2003) provide a significant challenge to the threshold and linear models because such data indicate that hormetic models are indeed more frequent than even the common threshold model, that such hormetic

findings conform to rigorous statistical criteria and are visually confirmed.

As noted above, to estimate the frequency of hormetic dose response within the toxicological literature, a specific database was created (i.e., Hormesis Database II–Frequency). Three journals (i.e., *Bulletin of Environmental Contamination and Toxicology*, *Environmental Pollution*, *and Life Sciences*) were evaluated which published many articles addressing ecologically and human oriented toxicology, thereby likely assuring the capacity for broad generalization. On the other hand, such findings are limited in their capacity to address specific endpoint hormetic frequencies such as those dealing with immune function, mutagenicity, teratogenicity, tumorigenicity, and others, although numerous examples of these endpoints are included in both hormesis databases (Calabrese and Baldwin, 2001a). Forty percent of the dose responses that satisfied the entry criteria also satisfied the evaluative criteria (Calabrese and Baldwin, 2001a). This 40% estimate could possibly change to as low as 25% or to as high as 75% depending on the degree of rigor built into the evaluation criteria.

The principal point is that hormetic findings are common in the peer-reviewed literature, independent of the journal published, the decade of the research, or the model, endpoint, or chemical class assessed. These findings suggested that the hormetic dose response model is a general phenomenon in the toxicological literature. Hormetic dose responses, therefore, should not be considered as exceptions to the rule or "paradoxical" phenomena. The principal reason for not observing hormetic dose–response relationships more often is usually due to many possible factors including the nature of the study design (e.g., the number of doses, dose spacing, and the lack of a temporal component), the background incidence of the endpoint selected for measurement as well as statistical power issues.

When this initial investigation by Calabrese and Baldwin (2001) estimated the frequency of hormetic dose response, it did not address other toxicological dose-response model frequencies. However, as noted above, a follow-up investigation assessed the occurrence of the hormetic vs. threshold model in the toxicological literature using the "hormesis frequency" database (Calabrese and Baldwin, 2003). The distribution of the responses of doses less than the NOAEL were not randomly distributed above and below control values as predicted by threshold model, but were non-randomly distributed in a manner highly consistent with the hormetic model. These two types of evaluation provide complementary perspectives on the occurrence of the hormetic and threshold models in the toxicological literature with the hormetic model clearly outperforming the threshold. These findings are very relevant to the issue of the selection of a default dose-response model for regulatory agencies such as EPA because the data were taken directly from three toxicologically oriented journals and the agents studied would be those generally viewed as environmental contaminants, many of which are currently regulated by EPA.

Application of dose-response model for endpoints of relevance to risk assessment

Endpoints used in risk assessment have often related to organ specific toxicities, tumor formation, behavioral alterations, respiratory irritation, and decrements in respiratory performance, and others. If the control disease incidences were low, it would not be possible to discern hormetic effects and the threshold model would likely predominate. This would be the case in chronic bioassays where the control disease incidence may be historically quite low for numerous tissues. Choice of animal model, therefore, can be a critical factor affecting the selection of the default model depending on their background disease incidence. The limitation in the capacity to demonstrate hormesis in a biological model with low background disease incidence does not have to be a barrier for accepting the hormetic model as generally applicable if hormesis could be reliably demonstrated for that same endpoint in other biological models with a high background incidence for that endpoint.

Capacity for false positive and negative estimates of dose-response model

In 2001, Calabrese and Baldwin reported estimates of absolute and relative false positive and negative values with respect to hormesis dose-response frequency. The estimates of false negatives and positives had low absolute values but were nearly 3-fold greater for false negatives than the false positives (9.7% vs. 3.5%). These findings suggest a relatively low net preference for false negative responses, thereby slightly underestimating the hormetic frequency rate. Given the low absolute false negative and positive rates, estimates of hormetic frequency appear stable.

How dose-response models impact hazard assessment study requirements

The traditional hazard assessment method (i.e., animal model, study design features such as sample size, number of doses, dose spacing, duration, and endpoints measured, etc.) is designed to provide information using a limited number of doses to estimate LOAELs and NOAELs to obtain RfDs for noncarcinogens or to model carcinogen responses at low doses. This hazard assessment information could be used by the hormetic default as with the other competitive models to provide risk estimates. Thus, it is not necessary to alter current hazard assessment practices to accommodate the hormetic default. This would only be necessary if it were desired to validate the hormetic model estimates in the sub-NOAEL region of the dose response.

The hormetic dose-response model is based on a large quantity of data that defines the quantitative nature of the

hormetic stimulatory response. These data provide the foundation to make toxicologically based predictions in the hormetic zone. Most ''government mandated'' hazard assessment study designs are not adequate to assess hormesis due to insufficient numbers of dosages and a general lack of a temporal component to the study. It is also not generally cost and time efficient to require major changes in the current hazard assessment protocol to attempt to answer the question of whether hormesis occurs in every assay. Based on the quantity and high quality of the data on hormetic dose responses, their quantitative dose-response features, and broad generalizability, acceptable predictions of biological responses below the NOAEL within the context of a default model framework can be made, and, if necessary, experimentally validated as noted above. Based on the data available, these predictions are expected to be superior to those offered by other competitive models (e.g., linear, threshold).

Reliability of estimating risk with the proposed dose-response model

No significant technical challenges exist in the application of methods of estimating possible risks and acceptable exposures based on the hormetic dose-response model for noncarcinogens and carcinogens. Several papers (Calabrese, 1996; Gaylor, 1998) have addressed how the hormesis concept could be applied to the derivation of reference dose values for noncarcinogens utilizing the traditional UF methodology by federal agencies such as EPA. Calabrese and Baldwin (1998) also explored how the concept of hormesis has the potential to affect NOAEL derivation especially in the benchmark dose (BMD) method. Sielken and Stevenson (1998) have provided guidance concerning how adoption of the hormesis concept could alter biostatistical modeling of low-dose cancer risks (Table 1).

Table 1
Implications of hormesis for quantitative risk assessment

Dose–response models need greater flexibility to fit the observed shape of the dose–response data; such models should not be constructed to be forced to always be linearly decreasing at low doses.
Hazard assessment evaluations need to incorporate greater opportunity to identify the hormetic portion of the dose–response relationship.
New dose metrics should be used that incorporate age or time dependence on the dose level rather than a lifetime average daily dose or its analog for a shorter time period.
Low-dose risk characterization should include the likelihood of beneficial effects and the likelihood that a dose level has reasonable certainty of no appreciable adverse health effects.
Exposure assessments should fully characterize the distribution of actual doses from exposure rather than just upper bounds.
Uncertainty characterizations should include both upper and lower bounds.
Risk should be characterized in terms of the net effect of a dose on health instead of a single dose's effect on a single disease endpoint (i.e., total mortality rather than a specific type of fatal disease).

Note. Adapted from Sielken and Stevenson (1998).

Capacity to validate estimates of risk

The hormetic dose-response model provides data in the observable range without reliance on the need for extrapolation procedures. This is a very important feature of the hormetic dose response because the hormetic curve has been demonstrable within the context of routine experimental studies.

Public health implications of risk assessment estimates

The hormetic model was more common than the threshold model in our assessment, suggesting that the hormetic model may occur 2.5 times more frequently than the threshold model. This raises the question of what are the implications of acceptance of the hormetic model over the threshold model when dealing with noncarcinogens. The first question one would have to resolve is whether the response below the NOAEL (i.e., hormetic stimulation) was considered beneficial, neutral effect, or adverse. If the response were considered potentially adverse, then there may be a need to derive a new ''NOAEL'' at the start of the hormetic zone. There are many situations in which the below NOAEL hormetic response could conceivably be harmful. These may include but not be limited to organ proliferation such as an increase in the prostate size resulting in reduced urine flow rate, immune alterations such as synthesis of auto-antibodies, endocrine alterations, and tumor cell or infectious agent proliferation. If such possibilities existed, the hormetic model could offer a distinct advantage over the under-predictive threshold model in protecting public health. With respect to possible neutral responses, the interpretation of the significance of the hormetic response could be made from the nature of the endpoint and a magnitude of the change. In such a case, it is possible that the traditional NOAEL would still be retained. If the sub-NOAEL response were likely to be beneficial, then the risk manager could be presented with a set of more complicated options that could consider balancing benefits vs. risks for various population subgroups (see Calabrese, 1996, and Gaylor, 1998, for a discussion of this issue). Regardless of the final disposition in these three hypothetical situations, the hormetic model offers considerably more insight and options than the traditional threshold model while starting from a considerably stronger toxicological foundation.

The hormesis dose-response model default concept, at least superficially, may appear to be more challenging for carcinogens than noncarcinogens. This is likely to be the case because of the heightened concern that society and regulatory agencies have with the cancer endpoint. Suggesting modifications in how carcinogens are assessed and regulated may not only be a data-driven process but also affected by less objective criteria that relate to societal customs and complex belief systems. In addition, data supporting the hormesis hypothesis that directly relates to

the process of carcinogenesis is much more limited than that available for noncarcinogenic endpoints. In fact, only 3–4% of the dose–response relationships satisfying the a priori criteria permitting entry into the Hormesis Database—I reflect mutational and cancer endpoints. In absolute terms, the number of such dose–response relationships is about approximately 250, a sizeable number although it is a very small percentage of the total entries into the database.

There has been a substantial research effort by Japanese investigators over the past decade that has specifically incorporated the concept of hormesis in the assessment of chemically induced liver cancer in the male F344 rat, a standard rodent model used by the U.S. National Toxicology Program in their chronic bioassay program. This activity has included evaluation of many tumor promoters such as phenobarbital and complete carcinogens such as DDT and has established the hormesis phenomenon in that model. In addition, these investigators have also undertaken substantial mechanism-related research to account for the U-shaped/hormetic findings of their investigations. This work is particularly noteworthy because it has demonstrated that the hormetic phenomenon could be reliably replicated, mechanistically explained, and then predictively applied to other agents and experimental settings which likewise demonstrate the hormetic dose response for endpoints which are part of the process of carcinogenesis including foci and tumor formation (Kinoshita et al., 2003; Kitano et al., 1998; Masuda et al., 2001; Sukata et al., 2002; Tsuda et al., 2003).

Of particular interest is that well-conducted chronic bioassays also demonstrate hormetic response for cancers in multiple organs including the lung (Nesnow et al., 1994; O'Gara et al., 1965; Prahalad et al., 1997), mammary gland (Broerse et al., 1987; Kociba et al., 1978), ovary (Ito et al., 1992), bone (White et al., 1993, 1994), pancreas (Hajri and Damge, 1998), thyroid (NTP, 1982), bladder (Downs and Frankowski, 1982), testes (Waalkes et al., 1988), and hematological (Maisin et al., 1988) system including leukemias. In the case of lung tumors, hormetic dose responses have been demonstrated for well-known carcinogens including some of the PAHs (Nesnow et al., 1994; Prahalad et al., 1997) and radiation (Ullrich and Storer, 1979). In particular, radiation studies have stood out because of the many animals employed in the various individual treatment groups, which at times exceeded several thousand (Ullrich and Storer, 1979). That is, the statistical power in such studies far exceeds what is typically seen in the normally viewed as substantial NTP chronic bioassays. However, hormetic responses to my knowledge have yet to be demonstrated for many organs/organ systems including the brain, colon, and skin, areas of key human interest. This lack of evidence of an hormetic response does not mean that hormetic responses do not occur with such tissues. Hormetic responses have been reported in these tissues although they have yet to show the hormetic response for endpoints that are essential components of the process of carcinogenesis.

A review of cancer-related studies with evidence of hormesis indicates that in the majority of cases, the investigators did not consider the possibility of an hormetic response in the design of the study. In fact, this is also case with investigations of noncarcinogenic endpoints demonstrating hormesis as well. This general lack of consideration in the design of the experiments has important implications for the capacity to assess hormetic dose responses.

In addition to the tumor/cancer endpoint studies, there have been a substantial number of investigations dealing with mutagens which suggest an hormetic response. The type of agents demonstrating such responses is quite diverse and includes environmental contaminants (e.g., cigarette smoke—Bonassi et al., 2003; DL-menthol—Kiffe et al., 2003; aniline—Sicardi et al., 1991; lignin derivatives—Mikulasova and Kosikova, 2003; butylated hydroxyanisole—Oh et al., 2001) and possible therapeutic agents (e.g., VM-26, VP16-213, and platin, Singh and Gupta, 1983).

Of particular interest with respect to hormesis and cancer is the recognition that hormetic effects are widely seen for a very broad range of immune endpoints, many of which may have important implications for the process of carcinogenesis (Calabrese, 2004). Furthermore, several investigations indicate that very low level exposures to radiation can be used in both preventive and therapeutic settings to minimize the metastasis of normally very aggressive injected lung tumor cells into experimental animals (Hashimoto et al., 1999; Sakamoto et al., 1997).

The general findings indicate that U-shaped dose–response relationships are common with respect to mutational events and cancer incidence. Most tumor types display U-shaped dose–response relationships in some, but not necessarily the majority, of studies. Although this situation would seem perplexing, it is actually what would be expected if one were to assume that there is likely to be some degree of variability among organs with respect to their susceptibility to injury and their capacity to repair such damage (Kojima et al., 1997, 1998a, 1998b). That is, it is likely that each organ system will display a unique dose–response relationship. However, the reality of the cancer bioassay is that because it is imposed on all tissues simultaneously, one would predict a diverse array of organ-specific dose responses. In addition, each organ will have a different background tumor incidence, which will affect the capacity to assess hormetic responses.

Final perspectives

The quantitative features of the dose–response relationships for endpoints inherent in the process of carcinogenesis and displaying evidence consistent with the hormesis hypothesis are similar to noncarcinogenic endpoints also

showing evidence of hormesis (Calabrese and Baldwin, 2001a, editors). This is the case with respect to the amplitude and the width of the hormetic response and its relationship to the NOAEL. Such consistency in the nature of noncarcinogenic and carcinogenic dose responses displaying hormesis represents an important and useful observation, suggesting a similar underlying biological strategy in the organism's response to the chemical/physical stressor agents under study. Furthermore, this general dose-response feature consistency with respect to hormesis between noncarcinogens and carcinogens suggests that adoption of an hormetic model with respect to interpreting the dose response in the low-dose zone may provide a toxicologically based means for harmonizing risk assessment procedures for noncarcinogens and carcinogens, a reconciliation which to date has not been achieved even after substantial efforts (Bogdanffy et al., 2001; Calabrese and Baldwin, 2001).

The striking consistencies in the features of the dose response across biological models, endpoints, and chemical/physical agents strongly support the generalizable nature of the hormesis concept but also suggests that the key component for understanding the dose response is the organism and the population sample under study more so than the agent under study. For regardless of the agent, the dose-response features remain very similar.

Based on the above summary of information, a conceptual case can be made for the adoption of hormesis as the default model for risk assessment and the possible harmonization of noncarcinogen and carcinogen risk assessment via use of the hormetic perspective. It represents a data-driven challenge to the current evaluative schemes to assess risks. Although the preponderance of evidence supports the consideration of hormesis as the default dose-response model for all types of endpoints including the present characterization of carcinogens and noncarcinogens, the current set of risk assessment procedures used by regulatory agencies, at the very least, should explicitly and comprehensively address the biological reality of hormetic dose–response relationships and recognize their capacity to enhance biological/toxicological understandings of dose–response relationships, something that has yet to be undertaken at the national and international levels.

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REVIEW

Hormetic mechanisms

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Abstract

This article provides the first extensive documentation of mechanisms of hormetic dose/concentration responses. The mechanisms selected were principally those mediated via receptor and/or cell signaling pathways. Mechanisms are reported for greater than 100 agents affecting nearly 400 dose/concentration responses from a wide range of chemical classes, affecting a broad range of cell types and endpoints. Regardless of the model (i.e. *in vitro* or *in vivo*), inducing agent, endpoint, or receptor/cell signaling pathway mediated mechanism, the quantitative features of the hormetic dose/concentration responses are similar, suggesting that the magnitude of the response is a measure of biological plasticity, within a broad range of biological contexts. These findings represent an important advance in the understanding of the hormetic dose/concentration response, its generalizability and potential biomedical applications, including drug discovery/efficacy assessment and the risk assessment process.

Keywords

Biphasic, cell signaling, dose-response, hormesis, hormetic, receptor-mediated mechanism, U-shaped

History

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Table of Contents

Introduction

The hormetic dose/concentration response has become the object of considerable investigation across the broad range of biological/biomedical disciplines concerned with dose/concentration response relationships (Calabrese, 2008; Calabrese & Baldwin, 2002; Mattson, 2008). This is supported by the observation that citations of the terms hormesis or hormetic in the peer-reviewed scientific literature have markedly increased over the past two decades (Figure 1). During this period evidence emerged that hormesis is highly generalizable, independent of biological model, endpoint measured, inducing agent and level of biological organization (e.g. cell, organ, organism). Furthermore, in direct comparison with other leading dose/concentration response models (e.g. threshold and LNT), the hormetic model demonstrated far

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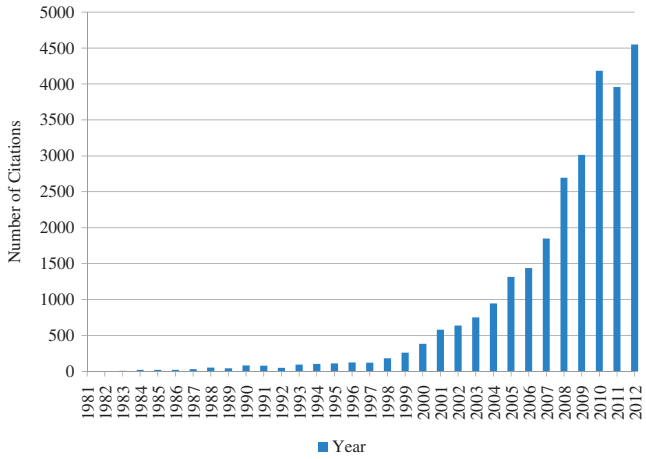


Figure 1. Citations of hormesis/hormetic in Web of Knowledge database.

more accurate predictions of low dose/concentration responses in multiple comparisons involving a wide range of biological models, endpoints, and inducing agents (Calabrese & Baldwin, 2001a; Calabrese et al., 2006, 2008, 2010). Despite these advances in the assessment of hormetic dose/concentration responses, there has been a need to better clarify the mechanistic foundations of the hormetic dose/concentration response. In 1977, Szabadi proposed that hormetic-like biphasic dose/concentration responses could occur by a single agonist, acting via two receptor subtypes that mediated/activated opposing stimulatory and inhibitory pathways. Stimulation is often mediated by receptors with high affinity for the agonist but with low capacity (i.e. relatively few receptors). In contrast, the inhibitory response typically occurs via receptors with lower agonist affinity while having greater receptor capacity. The proposal and documentation of Szabadi (1977) was supported and widely extended (Accomazzo et al., 2002; Alfonzo et al., 1998; Jarv, 1995; Jarv et al., 1995; Rovati & Nicosia, 1994).

In complementary fashion Stebbing (1982, 1998, 2011; Stebbing et al., 1984) suggested that hormetic dose/concentration responses represented a cybernetic feedback process that resulted in a modest overcompensation/net stimulatory response at low doses. The perspective of Stebbing was foreshadowed by earlier research by Townsend (1897), Branham (1929) and others which emphasized that overcompensation stimulation was a key component for understanding biphasic dose/concentration responses. The findings of Stebbing were subsequently supported by extensive documentation (Calabrese, 2001). Despite these conceptual and documented mechanistic developments, research on the hormesis concept continued to display a general lack of identifiable molecular targets such as receptors and/or signaling pathways, by which hormetic dose/concentration responses may be mediated.

The present article provides the first extensive evaluation of hormetic dose/concentration responses based on their mediation by cellular receptors and/or cell signaling pathways. The dose/concentration responses selected for evaluation are based upon the entry and evaluative criteria of the Hormesis Dose Response Database (Calabrese & Blain, 2005, 2009, 2011). The article provides evidence for hormetic mechanisms from several hundred dose/concentration-responses, using more than 100 agents (Tables 1 and 2), including endogenous agonists, drugs, industrial chemical agents and ionizing radiation, tested in a wide range of experimental models and affecting a large number of highly diversified biological endpoints. All experimental dose/concentration responses with identified hormetic mechanisms are given in Supplemental Data 1 (receptor mediated) and Supplemental Data 2 (cell signaling mediated).

Table 1.Agents that displayed hormetic dose/concentration response relationships mediated via a specific identified receptor.

Endogenous agonists
17β-estradiol
2-methoxyestradiol
α-thrombin
Advanced Glycation End-Products (AGE)
Allopregnanolone
Angiotensin II
CCK-8
β-Amyloid
Corticosteroid
Endothelin-1 and 3
Gonadotropin Releasing Hormone
Growth Hormone
Histamine
Hydrogen peroxide
IL-1B
IL-8
L-arginine
Lysophosphatidic acid
Marinobufagenin
Nitric Oxide
Noradrenaline
Ouabain
Oxytocin
Platelet activating factor (PAF)
Progesterone
Serotonin
Spermine
Sphingosine-1-Phosphate
Taurodeoxycholic acid
TGF-B
Thrombospondin-1 (TSP-1)
TNF
Drugs/toxins/natural products
Biochanin A
Buprenorphine
Daidzein
Dexamethasone
Genistein
Lambda-Cyhalothrin
Licorice-extracts-DMSO/EtOAc
MNNG
Nicotine
Opiate Agonists
Organochlorine Insecticides
Panxadiol glycoside
Phenylephrine
Pyrethroids
R(+)-WIN 55212-2
Simvastatin
Tributyl Tin
Xylazine

Receptor-mediated and cell signaling-mediated hormetic mechanisms

The conceptual formation of a receptor-based mechanism for hormetic-like biphasic dose/concentration response

Table 2. Agents that displayed hormetic dose/concentration response relationships mediated via a specific cell signaling mechanism.

Endogenous agents
Adenosine
Angiotensin II
Aracadonic Acid Metabolite-15d-PGJ2
Atrial Natriuretic Peptide
CCK-8
Epidermal Growth Factor
Epinephrine
ERK-2/p38/JNK
Estradiol-B
Hepatocyte Growth Factor
Hydrogen Peroxide
Hydrogen Sulfide
IFN-B
Leptin
LPS
Lysophosphatidic Acid
Lysophosphatidylcholine
Met-enkephalin
N-acetylcyteine (NAC)
Netin-1
Peptides, PACAP-27,28
Platelet Lysate
Prostaglandin E2
S100B
Serotonin
Somatostatin
TNF-a
Vitamin D3
Drugs/Toxins/Natural Products
17-AAG (17-allylamino-17-demethoxygeldanamycin)
Ac-SDKP
Arsenic
Atorvastatin
Auranofin
Azathioprine
Azide
B-Lapachone
Bisphenol A
Cadmium
Curcumin
Dehydroepiandrosterone (DHEA)
DNP-HAS/SIP
Ethanol
Ethanolamine
Fluoxetine
Fluvastin
Gamma Rays
Genistein
HMGb1
Jojoba Liquid Wax
Ligustilide (Z)
M6434
Menadione
Methamphetamine
Minocycline
Morphine
Naringin
Neoeriocitrin
Ouabain
Pentoxifylline
PDBu-12,13-Dibutyrate
Peroxynitrite
Phenazine
Phorbol ester (PMA)
Resveratrol
X-Rays

relationships by Szabadi (1977) set the stage for hormetic dose/concentration-response mechanistic advances that began in the 1980's and that have continued with increasing activity to the present. In the decade following Szabadi (1977), numerous receptors were identified that mediated biphasic dose/concentration responses (Calabrese & Baldwin, 2001a, 2001b). These examples displayed dose/concentration response relationships that conformed to the quantitative features of the hormetic dose/concentration response.

During the decades of the 1980s and 1990s advances occurred with respect to the development of antagonists for many receptors which were then used to evaluate whether hormetic dose/concentration-response mechanisms may be receptor based and/or mediated. While the synthesis, assessment and application of receptor antagonists for biphasic dose/concentration response mechanism evaluation became progressively stronger during the decade of the 1990's, similar developments occurred for cell signaling pathway mechanisms, except initially delayed by almost a decade. Figure 2 illustrates the occurrence by year of receptor-based and cell signaling-based pathway mechanisms of hormetic dose/concentration responses and the rapid development of the cell signaling pathway findings in recent years.

Criteria to evaluate the occurrence of receptor-mediated hormetic mechanisms

While full understandings of dose/concentration-dependent transition mechanisms remain to be achieved, considerable mechanistic knowledge has been developed for a large number of hormetic dose/concentration responses. In the present assessment three *a priori* entry criteria needed to be satisfied for receptor mechanism evaluation: 1) a reproducible biphasic dose/concentration response with quantitative features of an hormetic dose/concentration response; 2) the dose/concentration response stimulation had to be hypothesized to be mediated by a specific receptor; and 3) this hypothesis needed to be tested and demonstrated by a specific antagonist for this receptor.

With respect to evaluation criteria that defined a mechanism, the minimum criteria required the testing of an antagonist against at least one agonist concentration in the stimulatory (i.e. hormetic) zone. If the stimulatory response was blocked by the receptor antagonist then it was concluded that the hormetic stimulation was mediated via this receptor. The available studies displayed antagonist/agonist interactions ranging from one agonist concentration tested to having all agonist concentrations in the stimulatory zone tested. In addition, inhibitory agonist dose(s)/concentration(s) were tested against the same and/or other receptor antagonist; the results of the assessment of inhibitory agonist dose/concentration did not affect a judgment on the presence or absence of an hormetic mechanism. In contrast with the low dose/concentration stimulation, high dose/concentration inhibition was not consistently evaluated with a receptor antagonist in the published papers. However, there are numerous cases in which such testing did occur, leading to an assessment of mechanism in the stimulatory and inhibitory zones. When such high dose/concentration testing was performed, the number of agonist doses/concentrations

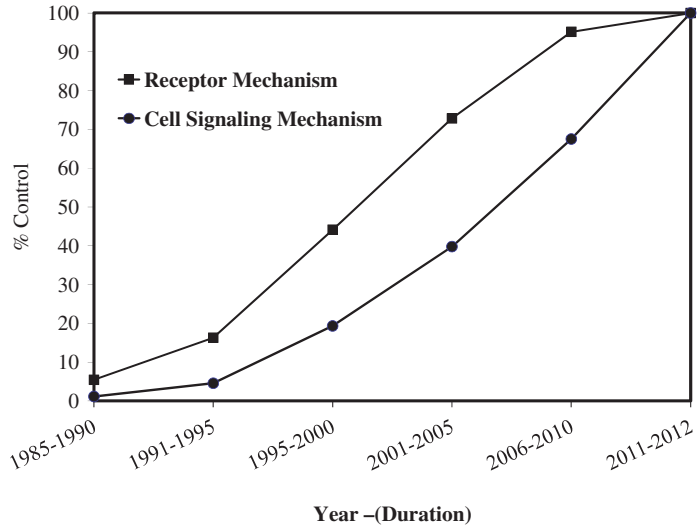


Figure 2. Receptor mechanism/cell signaling mechanism reported in scientific literature by year.

Year Duration	Receptor Mechanism(%)	Cummulative Average	Cell Signaling Mechanism(%)	Cummulative Average
1985-1990	5/92 (5.4)	5.4	1/88 (1.1)	1.1
1991-1995	10/92 (10.8)	16.3	3/88 (3.4)	4.5
1996-2000	26/92 (28.3)	44.1	12/88 (14.8)	19.3
2001-2005	26/92 (28.3)	72.8	18/88 (20.5)	39.8
2006-2010	21/92 (22.8)	95.1	42/88 (47.7)	67.5
2011-2012	4/92 (4.3)	99.9	11/88 (12.5)	99.9

tested ranged from only one dose/concentration tested to all doses/concentrations tested. These different experimental strategies lead to the occurrence of several mechanistic hypotheses concerning hormetic dose/concentration responses.

Characterization of receptor-mediated hormetic mechanisms that address stimulatory and inhibitory responses

Hormetic dose/concentration response mechanistic assessment #1

This occurs when the receptor antagonist blocks low dose/concentration stimulation and the high dose/concentration inhibition. In such situations both the stimulatory and the inhibitory responses are mediated by the same receptor (Figure 3A).

Hormetic dose/concentration response mechanistic assessment #2

This occurs when the receptor antagonist blocks the low dose/concentration stimulation but not the high dose/concentration inhibition. Furthermore, a second and distinct receptor-mediated mechanism not involving the agonist receptor mediating the low dose/concentration stimulation has been identified that mediates the high dose/concentration inhibitory response. In such circumstances the biphasic dose/concentration response is mediated by two separate mechanisms (Figure 3B).

Hormetic dose/concentration response mechanistic assessment #3

This occurs when the receptor antagonist blocks the low dose/concentration stimulation but not the high dose/concentration inhibition. In these situations the stimulatory response at low doses/concentrations is mediated by the agonist receptor, but the dose/concentration inhibition mechanism is unknown (Figure 3C).

Criteria to evaluate cell signaling-mediated hormetic mechanisms that address stimulatory and inhibitory responses

There is a complex and integrative array of signal transduction pathways that mediate hormetic stimulatory responses. Figure 4 provides a partial description of these pathways, their relationship to the cell membrane, membrane receptors, and partial sets of inter-connections among these signaling pathways. The experimental approach employed for the assessment of hormesis via cell signaling follows the same strategy as with receptor mediated mechanisms including the same entry and evaluative criteria. Pathway inhibitors (Grande & Lopez-Novoa, 2008) are used to assess whether the hormetic stimulation is mediated by specific cell signaling

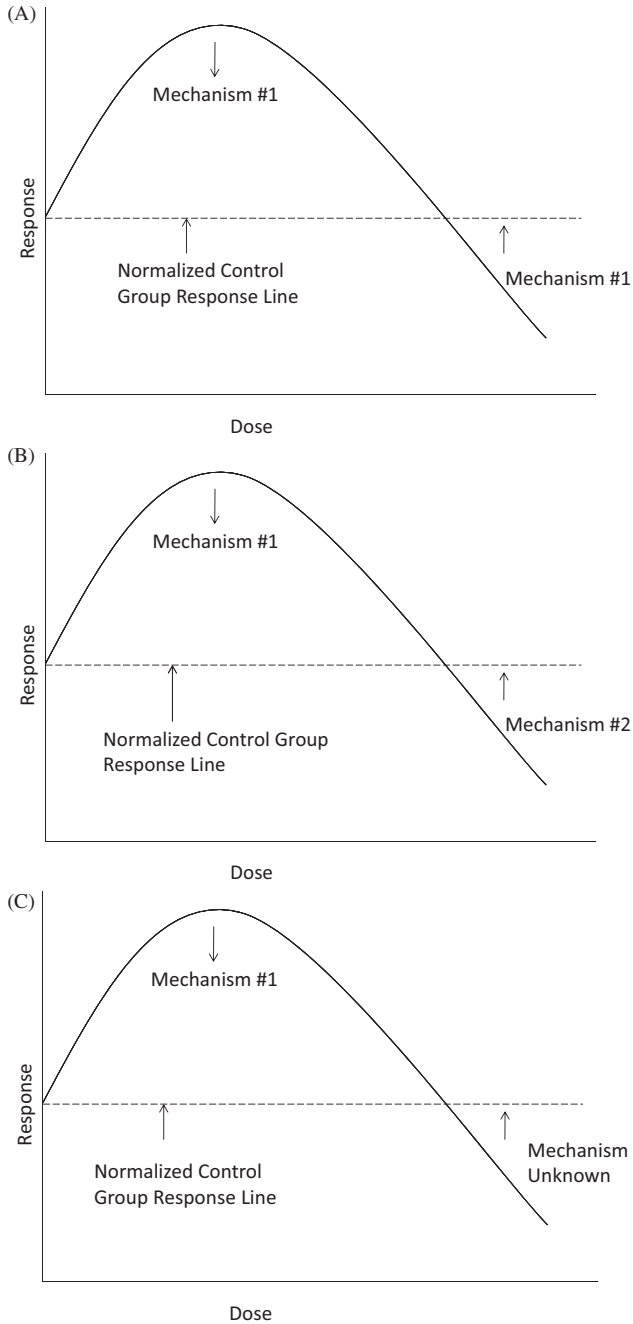


Figure 3. (A) Receptor-Mediated/Cell Signaling Pathway Mediated Hormetic Response. Mechanistic Assessment #1: Receptor antagonist (or cell signaling pathway inhibitor) blocks low (↓) dose/concentration stimulation and high (↑) dose/concentration inhibition. (B) Receptor-Mediated/Cell Signaling Pathway Mediated Hormetic Response. Mechanistic Assessment #2: Receptor antagonist blocks (or cell signaling pathway inhibitor) the low (↓) dose/concentration stimulation but not the high (↑) dose/concentration inhibition. A second mechanism has been identified that mediates the high (↑) dose/concentration response. Low dose/concentration stimulation is blocked by receptor antagonist (or cell signaling pathway inhibitor) but high dose/concentration inhibition is blocked by different mechanism. (C) Receptor-Mediated/Cell Signaling Pathway Mediated Hormetic Response. Mechanistic Assessment #3: Receptor antagonist (or cell signaling pathway inhibitor) blocks low (↓) dose/concentration stimulation but not high dose/concentration inhibition. The inhibitory mechanism is not known.

pathways. If blockage of a specific cell signaling pathway prevents the hormetic stimulation this is evidence that the hormetic dose/concentration response is mediated by this signaling pathway. Some experiments assess multiple signaling pathways; this can be used to assess the relative significance of each pathway and their possible interactions involved in the hormetic stimulation. As with receptor mediated hormetic responses, inhibitory agonist doses/concentrations were tested against the same signaling pathway(s) inhibitors.

There are three types of hormetic dose/concentration response mechanisms for signaling pathways that follow the same scheme for receptor mediated mechanisms. These are based on whether the same pathway affects both stimulation and inhibition, only stimulation or whether there are distinct mechanisms for stimulation and inhibition.

For some of the dose/concentration-responses demonstrating the hormetic response unique alternative mechanisms have been determined that are not mediated by either receptor or signaling pathways. These examples are addressed separately.

Hormetic mechanism findings

There is a broad range of agents for which hormetic mechanisms have been determined using the present criteria. Agents acting via receptor mediated processes are given in Table 1 and those acting via cell signaling pathways are given in Table 2. Only a small number of agents (i.e. 17β-estradiol, angiotensin II, CCK-8, hydrogen peroxide, lysophosphatidic acid, and TNF) have mechanistic determinations for both receptor and signaling pathways based on the entry and evaluative criteria applied in this assessment.

Some extensively studied agents have hormetic mechanisms determined in a broad range of cell types and for diverse endpoints. For example, estrogen has hormetic mechanisms established in six experimental systems for cancer cell proliferation, in four non-cancer cell types for cell proliferation, and for seven other endpoints (Table 3). Multiple hormetic mechanisms for various cell types and endpoints have also been reported for agents such as histamine, nicotine, glucocorticoid, adrenergic pathway agents, nitric oxide, among others (Table 3).

Multiple study designs have been used in the experimental assessment of hormetic mechanisms for receptor and cell signaling pathway mediation (Table 4-first two columns on left). While each of these designs can lead to a decision on hormetic mechanisms, the broad range and diversity of the experimental features affects the capacity to derive mechanistic insights of hormetic dose/concentration responses. For example, there is considerable variation with respect to the number of agonist doses/concentrations tested against receptor antagonists at stimulatory and/or inhibitory doses/concentrations.

Approximately 50% of the dose/concentration-responses with receptor mediated and cell signaling pathway mediated mechanisms have only the low dose/concentration stimulation agonist tested (Table 4). In these dose/concentration responses the high dose/concentration affected a marked inhibitory response but there was no testing for a possible mechanism for the high dose/concentration response (e.g. inhibition). The second most commonly assessed response for mechanisms were those dose/concentration responses for which there was both a low dose/concentration stimulation

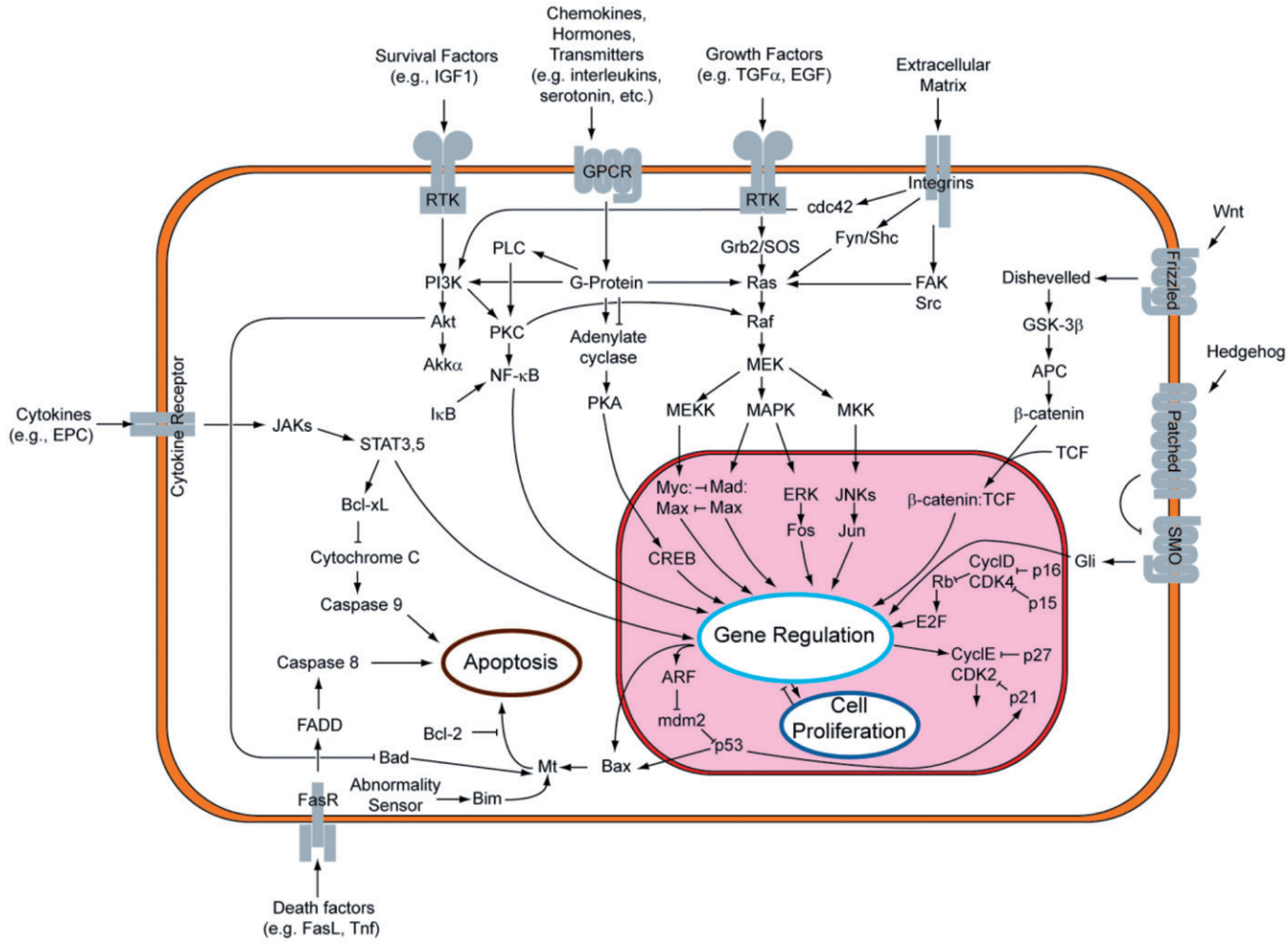


Figure 4. Signal transduction pathways. (*Source:* commons.wikimedia.org, file in public domain).

and a high dose/concentration inhibition and for which there was at least one agonist dose/concentration in the stimulatory and inhibitory zones tested against an antagonist. These two combined groups comprise about 63% of the dose/concentration responses for both the receptor and cell signaling pathways. The third most common dose/concentration response tested for the receptor mediated mechanism (13.3%) and fourth most common for the cell signaling pathway mechanism (10.5%) had a dose/concentration response with both low dose/concentration stimulation and high dose/concentration inhibition and with the entire set of agonist doses/concentrations tested against antagonists.

There were some dose/concentration responses in which the high agonist dose/concentration did not display evidence of a high dose/concentration inhibition but only a return to control group values at the highest dose(s)/concentration(s) tested. This may have occurred due to an inadequate dose/concentration range tested at the high end or because no toxicity/inhibition was induced. Within this grouping some dose/concentration responses were tested for all agonist doses/concentrations while the entire range of agonist doses/concentrations was not tested for others. These dose/concentration responses together comprise about 10–18% of the dose/concentration responses in the receptor and cell signaling pathway groups. Finally, approximately 6–8% of the biphasic dose/concentration responses were alternative mechanisms without a receptor or cell signaling pathway for the hormetic stimulation. Table 5 summarizes the occurrence of cell signaling pathway mediated mechanisms and the relationship between the integrated endpoints measured and underlying cell signaling responses.

Specific hormetic mechanisms

Receptor-based hormetic mechanism #1

Numerous examples exist in which both low dose/concentration stimulation and high dose/concentration inhibitory effects were mediated via the same receptor (i.e. Mechanism #1; Figure 5). For example, corticosterone-induced prolactin production and inhibition in MTT/SM cells (Yokoyama et al., 2008; Figure 5.1) was blocked via the antagonist mifepristone. The biphasic (stimulation and inhibition) dose/concentration responses of dexamethasome (DEX) on the growth of neuroepithelial tumor cells were blocked via the glucocorticoid antagonist RU38486 (Kawamura et al., 1998; Figure 5.2). The biphasic effects of estradiol on the expression of the NOS isoform in N-SH-cell extracts (Xia & Krukoff, 2004; Figure 5.3) was blocked by ICI 182,780. The use of a single receptor to mediate both the stimulatory and inhibitory effects of the biphasic/hormetic dose/concentration response

Table 3. Agents displaying hormesis for which there is a well-defined mechanistic basis.

Agonist agents inducing hormesis	Hormetic endpoint	Experimental model
Estrogen	Cancer cell proliferation	–Prostate (LNCaP) –Colon (MC-26) –Pituitary (GH3) –Breast (MCF-7, others) –Intestine (CaCo) –Capillary sprouting in *in vitro* tumor angiogenesis model
Estrogen	Non-cancer cell proliferation	–Vascular smooth muscle –Endothelial cells –Bone marrow cells –Adipocytes
Estrogen	Other endpoints	–Bone cell metabolism –-ALP expression –-Calcium uptake –Nitric oxide synthase expression –Macrophage induction/release of nitric oxide, TNF-α, VEGF –Transposin-1 regulation –Spontaneous cytokine secretion in healthy post menopausal women –Nodules in osteoprogenitor cells
Histamine	Multiple endpoints (see experimental model column)	–Fibroblast – Multiple Models –-Proliferation –-Migration –Thymidine uptake into normal lung fibroblasts –Progesterone production by MA-10 cells (murine Leydig cells) –Testosterone production from Leydig cells/macrophages
Nicotine	Cell proliferation	–Osteoblast Proliferation –-Human, primary –Endothelial cell proliferation
Glucocorticoids	Cell Proliferation, Prolactin production-MTT/SM cells	–Neuroepithelial tumor (human) cell growth – dexamethazone –Keratocyte proliferation (human) –estrogen-induced mammotrophic tumor –Neuronal mitochondrial function – oxidation parameters –MCF-7 cells
Adrenergic pathway agents	Cell Proliferation, Renin release – rat juxtaglomeralar cells/noradrenaline, Na/K pump activity – human mesenteric arteries/ouabain marinobufagenin	–Rat thymocyte proliferation/xylazine –Rat spleen cell proliferation/xylazine – -Thymidine incorporation in fibroblast DNA/phenylephrine in neonatal foreskin –
Thrombospondin-1	Cell Migration	–Endothelial cells (BAEC, HUVEC, Endo742)
Thrombin Receptor (PAR-1)	Cell Proliferation	–Murine melanoma cell proliferation (B16F10) –Human colon adenocarcinoma cell proliferation (HCT8 cells)
Progesterone	Cell Proliferation	–Normal human ovarian epithelial cell proliferation –Human ovarian tumor cells (OVCA 420 cells) proliferation
Opioids	Cell Proliferation, Myelin basic protein levels	–Mouse lymphocyte proliferation –Sprague-Dawley rat
Oxytocin	Infarct Size/Myocardial Infarction; Learning/Memory Retention	–Male Sprague-Dawley rats; – Male Swiss mice
Cannabinoids	Neuronal Viability	Cultured Sprague-Dawley neurons
Platelet-Derived Growth Factor	Cell Proliferation	Prostate stromal cell
PAF	cAMP Production; Production of TNF-a – IL-1	– Guinea pigs; – Rat alveolar macrophage
Gonadotropin-Releasing Hormone (GnRH)	GnRH mRNA Levels; Synthesis of mRNA	– Ovarian cancer cell OVCAR-3 – GnRH receptor in HOSE cells
CCK-8	Cell Proliferation	–Pancreatic tumor cells
IL-1B	Cell Proliferation	Human pancreatic islet
TNF	Intracellular Ca^{2+}	Cardiomyocytes
TGFB	Cell Proliferation; Cell Migration	–Mouse osteoclast-like cells –Epithelial cells (EC-6 cells – normal rat intestinal cells)
Nitric oxide	Migration; Cell Proliferation	– IPEC-J2 cells

(*continued*)

Agonist agents inducing hormesis	Hormetic endpoint	Experimental model
		–Retinal microvascular endothelial cells (RMEC) –Human bronchial epithelial cells –Immortalized human bronchial epithelial cell line –Murine macrophages –Endothelial cells (BAEC/HUVEC)
5-HT	Cell Proliferation	–Cultured heart cells –Vascular endothelial cells of dogs
NMDA Receptor Agonist	Survival	Neurons of fetal rats
Endothelin-3	Migration	Rabbit neutrophils
IL-8	JAK3 Activity	Human neutrophils
Panaxadiol glycoside	Neuronal Differentiation	Neuronal stem cells (mouse neural stem cells)
Angiotensin II	Migration	Human aortic smooth muscle cells
Growth Hormone	Lipogenesis	Primary rat adipocytes
Sphingosine-1-phosphate (SPP)	Chemotaxis	–Human embryonic kidney cell (HEK 293)
LPA (lysophosphatidic acid)	RNA/Protein Synthesis	Cardiac fibroblast
Advanced Glycation Endproducts	Cell Proliferation	Mesangial cell (young Wistar rats)
Amyloid β1	Long Term Protentiation/B-Amylase	Mouse hippocampus
Ouabain	Cell Proliferation; Na/K Pump Activity	–Human mesentary arteries –rat vascular smooth muscle
Biochanin A	Cell Proliferation	T47D cells
Daidzein	Adipocyte Activity	–Osteoprogenitor cells
		–-K5483 cells
Genistein	Cell Proliferation	CaCo 2B Be cells
Genistein	ALP Activity	K5483 cells
Buprenorphine	Myelin Basic Protein Levels	Sprague-Dawley rat
Dexamethasone	Na+-K+-ATPase Activity	Bovine corneal endothelial cells
Phenylephrine	Cell Proliferation	Neonatal foreskin
Simvastatin	Cell Proliferation	Retinal microvascular endothelial cells
Xylazine	Cell Proliferation	Spleen cells
Lambda-Cyhalothrin (LCT)	Cell Proliferation	MCF-7
MNNG	Chemotaxis	Rabbit peritoneal neutrophils
Organochlorine Insecticides	Cell Proliferation	MCF-7 cells
Other Chemicals: Pyrethroids	Cell Proliferation	MCF-7 cells Receptor: Estrogen

Table 4. Dose/concentration response features (i.e. stimulation/inhibition) for receptor and cell signaling mediated responses and mechanism assessment.

	% of Total Receptor Mediated	% of Cell Signal Mediated	Average Receptor/Cell Signal Mediated Values
Low dose/concentration stimulation (LDS) – high dose/concentration response (HDR) does not go below control value – entire dose/concentration range assessed	5 (5.0)	0 (0)	5 (2.7)
LDS – HDR does not go below control value – entire dose/concentration range not tested	11 (10.9)	18 (21.7)	29 (15.8)
LDS – HDR goes below control value – entire dose/concentration range tested	14 (13.8)	4 (4.8)	18 (9.8)
LDS – HDR goes below control value – only low stimulatory dose(s)/ concentration(s) tested	45 (44.5)	50 (60.2)	95 (51.6)
LDS – HDR goes below control value – both low and high dose(s)/ concentration(s) tested	18 (17.8)	8 (9.6)	26 (14.1)
LDS – HDR goes below control value – only high dose(s)/concentration(s) tested	1 (1.0)	0 (0)	1 (0.5)
Alternative Mechanisms (non-receptor/non-signaling pathways)	7 (6.9)	3 (3.6)	10 (5.4)
Totals	101 (99.9)	83 (99.9)	184 (99.9)

Table 5. Cell signaling pathway evaluations: Comparison of magnitude of signaling pathway as compared to the integrative cellular response.

Reference	Agent	Model	Endpoint for integrated response	Signaling endpoint Marker	Signaling pathway response (SPR)	Integrative cellular response (ICR)	SPR/ICR ratio
Ranzato et al., 2010	Platelet lysate (PL)	Endothelial cell (repair assay)	Crystal Violet (CV)/Neutral Red Uptake (NRU)	pERK1/2 pAkt	Exposure: At 20% PL pERK1/2 (175%) pAkt (300%)	CV (120%) NRU (125%)	pERK1/2 ÷ CV = 1.46 pERK1/2 ÷ NRU = 1.40 pAkt ÷ CV = 2.50 pAkt ÷ NRU = 2.40
Lelievre et al., 1998	PACAP-27 PACAP-28	Neuroblastoma cell line (mouse neuro2a)	Cell proliferation (CP) [tetrazolium-derived colorimetric assay (MTS)]	pERK1/2	Exposure 1: 10^{-13} M PACAP-27 pERK1/2 (180%) Exposure 2: 10^{-13} M PACAP-28 pERK1/2 (183%)	PACAP-27CP (110%) PACAP-28CP (110%)	PACAP-27 pERK1/2 ÷ PACAP-27CP = 1.64 PACAP-28 pERK1/2 ÷ PACAP-28CP = 1.66
El Touny and Banerjee, 2009	Genistein	PC3 cells/prostate cell	Proliferation (measured by trypan blue assay)/invasion (invasion channels, and cell straining)	pAkt OPN protein levels	Exposure: 500 nmol/L pAkt (350%) OPN protein (350%)	# of cells (150%) Relative invasion level (220%) Relative MMP-9 activity (220%)	pAkt ÷ # of cells = 2.33 pAkt ÷ Invasion level = 1.59 pAkt ÷ MMP-9 = 1.59 OPN ÷ # of cells = 2.33 OPN ÷ Invasion level = 1.59 OPN ÷ MMP-9 = 1.59
Ranzato et al., 2009a	HmGb1	HaCaT keratinocytes	NRU (based on incorporation of NR into lysosomes of viable cells)	pERK1/2	Exposure: 10 nM pERK1/2 (150%)	NRU (210%)	pERK1/2 ÷ NRU = 0.71
Tian et al., 2009	Ouabain	Pig kidney epithelial cells (LLC-PK-1)	CP (MTT assay)	pAkt	Exposure 1: 1 nM pAkt (135%) Exposure 2: 10 nM pAkt (150%)	CP (150%) CP(155%)	pAkt ÷ CP = 0.9 pAkt ÷ CP = 0.97
Barca et al., 2010	IFN-B	Astrocyte/fetal Sprague Dawley rat	CP (neubauer counting chamber using BrDU)	pAkt	Exposure: 2.0 ng/ml pAkt (275%)	CP (175%)	pAkt ÷ CP = 1.57
Nguyen et al., 2007	Ouabain	Kidney cell proliferation (ADPKD)	CP (MTT)-18 experiments/6 replicates/experiments using 8 different kidneys	pERK1/2	Exposure: 10^{-8} M pERK1/2 (1150%)	CP (140%)	pERK1/2 ÷ CP = 8.2
Aydemir-Koksoy et al., 2001	Ouabain	Saphenous VSMC canine	DNA synthesis	pMAPK1/2	Exposure 1: 1 nM pMAPK1/2 (200%) Exposure 2: 10 nM pMAPK1/2 (100%)	DNA synthesis (125%) DNA synthesis (80%)	pMAPK1/2 ÷ DNA Syn = 1.60 pMAK1/2 ÷ DNA syn = 1.25
Wang et al., 2006	Genistein	RWPE-1 non-prostate cell mode	CP (MTT assay)	pERK1/2	Exposure: 10 umol/L pERK1/2 (135%)	CP (160%)	pERK1/2 ÷ CP = 0.84
Liang et al., 2011	X-rays	Rat mesenchymal stem cells	CP (MTT assay)	pERK1/2 pMEK pC Raf	Exposure: 75 mGy pERK1/2 (475%) pMEK (410%) cRAf (275%)	CP (120%)	pERK1/2 ÷ CP = 3.95 pMEK ÷ CP = 3.41 cRAf ÷ CP = 2.29
Kim et al., 2008	Epinephrine	Mouse embryonic skin cells	Cell number Chemocytometer	pERK1/2	Exposure: 10^{-6} M pERK1/2 (~175%)	CP (211%)	pERK1/2 ÷ CP = 0.62
Mantha and Jumarie, 2010	Cadmium (Cd)	Human enterocytic-like $CaCo_2$ cells	CP (MTT assay)	pERK1/2 p38 pJNK	Exposure: 10 uM pERK1/2 (400%) p38 (1,300%) pJNK (190)	CP (200%)- MTT	pERK1/2 ÷ CP = 2.0 p38 ÷ CP = 6.50 pJNK ÷ CP = 9.5

Liu et al., 2008	DHEA	Bovine aortic endothelial cells	CP (MTT assay)	ERK1/2 (p90 RSK) Nuclear pERK1/2	Exposure: 1 nM ERK1/2 (p90 RSK) (200%) Nuclear pERK1/2 (200%)	CP (135%)	ERK1/2 ÷ CP = 1.48 NpERK1/2 ÷ CP = 1.48
Wang et al., 2009	Netrin-1	Tubular epithelial cell of immortalized mouse kidney cell	CP (MTT assay)	pERK1/2 pAkt	Exposure: 100 ng/ml pERK1/2 (320%) pAkt (400%)	CP (128%)	pERK1/2 ÷ CP = 2.50 pAkt ÷ CP = 3.12
Jiang et al., 2009	Cd	Human embryo lung fibroblast	CP (MTT assay)	pERK1/2 pJNK p38	Exposure: 1 Umol/L pERK1/2 (225%) pJNK (225%) p38 (100%)	CP (150%)	pERK1/2 ÷ CP = 1.50; pJNK ÷ CP = 1.50; p38 ÷ CP = 0.66
Wartenberg et al., 1999	H_2O_2	Human prostate tumor cells (DU-145)	Growth of tumor speroids, calculate volume	pJNK pERK1/2 pMEK1/2	Exposure: 1 uM pJNK (225%) pERK1/2 (250%) pMEK1/2 (350%)	Spheroid growth (SG) (238%)	pJNK ÷ SG = 0.945; pERK1/2 ÷ SG = 1.05; pMEK1/2 ÷ SG = 1.47
Zang et al., 2009	Cd	Human breast cancer cells (T47-D)	DNA synthesis	pERK1/2	Exposure 1: 1 nM pERK1/2 (350%) Exposure 2: 10 nM pERK1/2 (1,500%)	DNA syn (135%) DNA syn (can plot entire DR) (137%)	pERK1/2 ÷ DNA syn = 2.59 pERK1/2 ÷ DNA syn = 10.94
Ranzato et al., 2011	JL wax (JLW)	HaCaT keratinocytes, human dermal fibroblast monolayers	Scratch wound healing	pAkt pERK1/2	Exposure : 0.5 JLW HaCaT pAkt (400%) pERK1/2 (200%) p38 (750%) Fibroblast pAkt (275%) pERK1/2 (400%) p38 (600%)	HaCaT Healing (425%) Fibroblast Healing (250%)	pAkt ÷ healing = 0.94 pERK1/2 ÷ healing = 0.47 p38 ÷ healing = 1.76 pAkt ÷ healing = 1.1; pERK1/2 ÷ healing = 1.6; p38 ÷ healing = 2.4
Cai et al., 2007	Hydrogen sulfide	RF/6A endothelial cells	CP/BrdU assay)	pAkt	Exposure: 10 umol/L pAkt (200%)	CP (115%)	pAkt ÷ CP = 1.74
Ranzato et al., 2009b	Platelet Lysate (PL)	$CsCl_2$ mouse myoblast	CV/wound closure	pERK1/2 p38 pAkt	Exposure: 20% PL pERK1/2 (450%) p38 (150%) pAkt (270%)	CV (160%)	pERK1/2 ÷ CV = 2.8 p38 ÷ CV = 0.93 pAkt ÷ CV = 1.68
Chueh et al., 2001	Ouabain	Human prostate smooth muscle	CP (MTT assay)	pERK1/2	Exposure: 0.1 nM pERK1/2 (300%)	CP (116%)	pERK1/2 ÷ CP = 2.58
Liu et al., 2010	Arsenic (trivalent)	MCF-7 10A (non-cancer cell line)	CP (MTT assay)	p38 pAkt pERK1/2	Exposure: 0.1 uM p38 (150%) pAkt (150%) pERK1/2 (130%)	CP (125%)	p38 ÷ CP = 1.25; pAkt ÷ CP = 1.20; pERK1/2 ÷ CP = 1.04
Wang et al., 2011	17AAG	Murine neural progenitor cells	CP (number of live cells present)	GSK3B Phosphorylate	Exposure: 10 nM GSK3B (250%)	CP (160%)	GSK3B ÷ CP = 1.56
Bouskine et al., 2009	Bisphenol A	JKT-1 cells	CP (cell counted in a hemocytometer	pERK1/2	Exposure: 10^{-9} M pERK1/2 (215%)	CP (130%)	pERK1/2 ÷ CP = 1.65

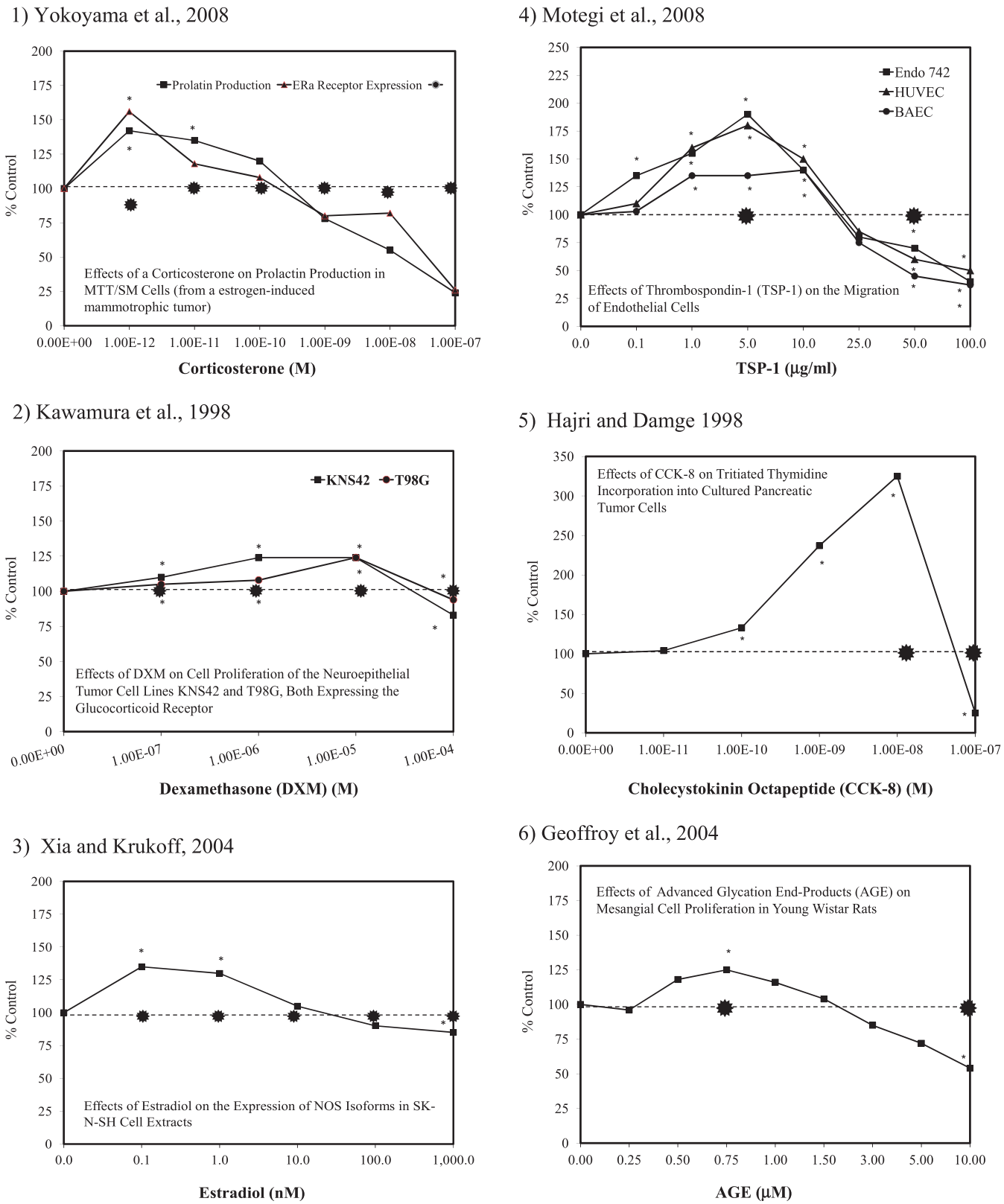
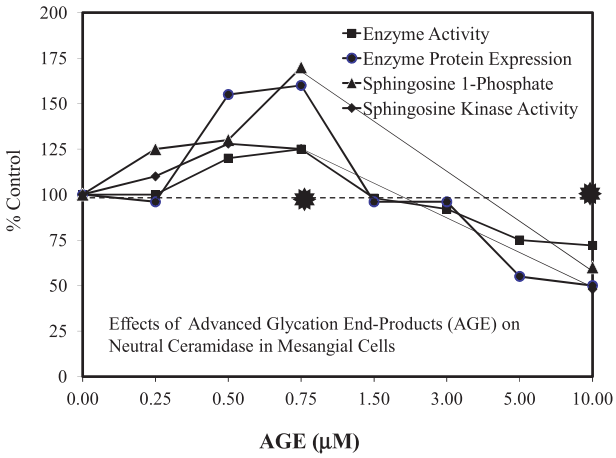


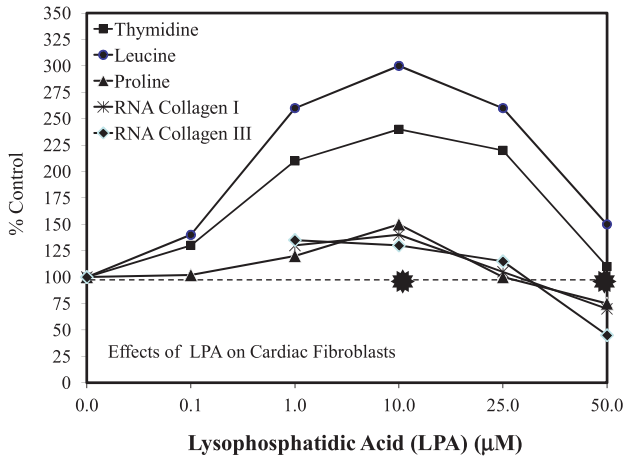
Figure 5. Receptor Mediated Mechanism – Mechanism #1. Examples of hormetic dose/concentration responses with a receptor-mediated mechanism. Stimulatory and inhibitory responses are mediated by the same receptor. Each hormetic response in this Figure indicates (✷) where the agonist was tested against an antagonist. The (✷) designation also indicates the response (%) for that agonist/antagonist interaction. For a more detailed explanation for the receptor mediated mechanism refer to Supplemental Data 1 for all graphs in Figure 5.

has also been reported for thrombospondin-1 (TSP-1; Figure 5.4; Motegi et al., 2008); CCK-8 (Figure 5.5; Hajri & Damge, 1998); advanced glycation end products (AGE; Figures 5.6, 5.7; Geoffroy et al., 2004); TNFα (Figure 5.8; Amadou et al., 2002); phenylephrine (Figure 5.9; Sterin-Borda et al., 2007); lysophosphatidic acid (LPA; Figure 5.10; Chen et al., 2006); and gonadotropin-releasing hormone (Figures 5.11, 5.12; Kang et al., 2000a,b).

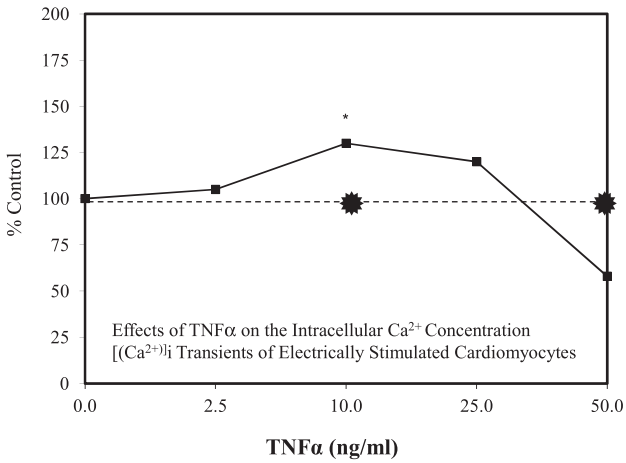
7) Geoffroy et al., 2004



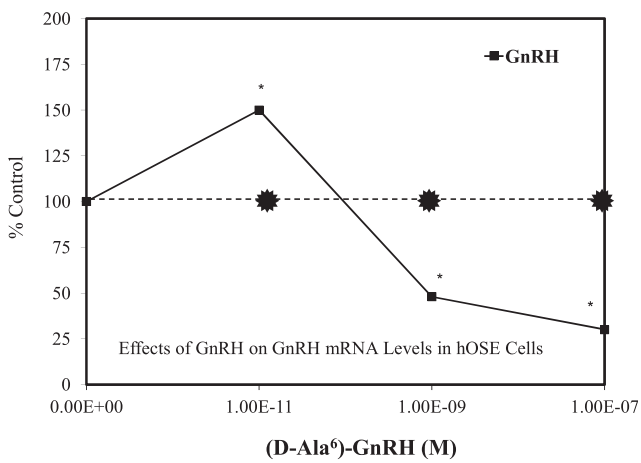
10) Chen et al., 2006



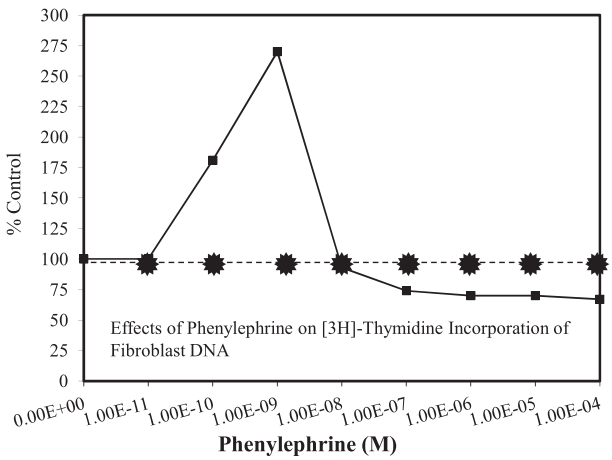
8) Amadou et al., 2002



11) Kang et al., 2000



9) Sterin-Borda et al., 2007



12) Kang et al., 2000

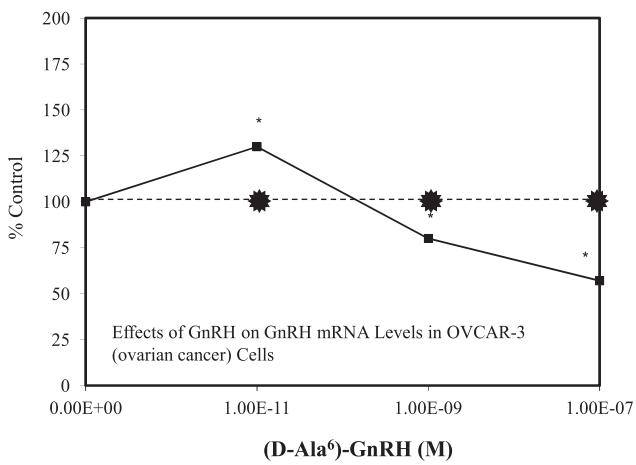


Figure 5. Continued.

Receptor-based hormetic mechanism #2

Noradrenaline induced low dose/concentration stimulation was blocked via a β-adrenoreceptor antagonist while its high concentration inhibitory response was blocked by an α-adrenoreceptor antagonist (Figure 6). This biphasic dose/concentration response was therefore mediated by different receptor subtypes (Figure 6.1; Takagi et al., 1992). A similar case is seen for endothelin-3 where the biphasic chemotactic migration was mediated by two receptor

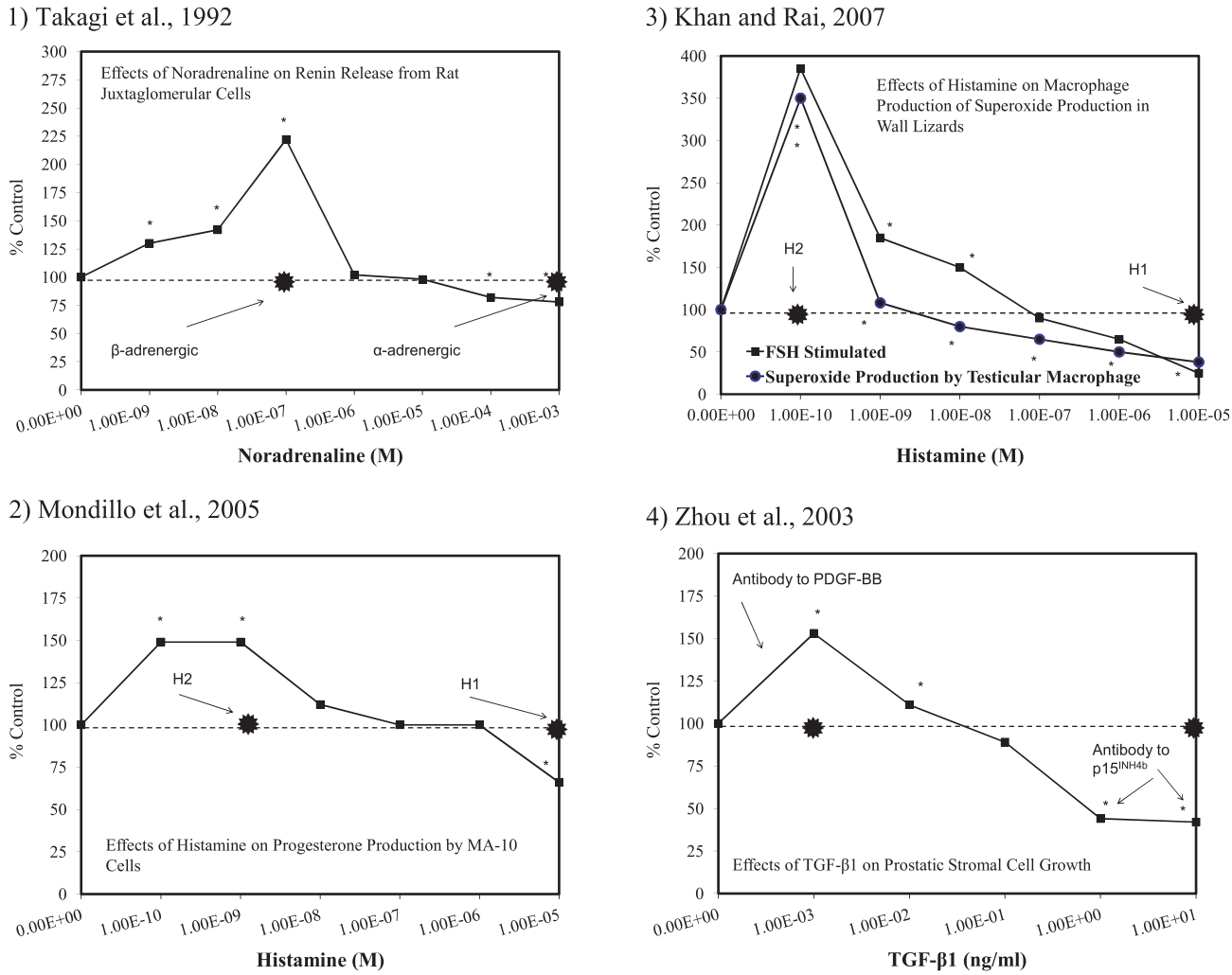


Figure 6. Receptor Mediated Mechanism – Mechanism #2. Examples of hormetic dose/concentration response with a receptor mediated mechanism. The stimulatory and inhibitory responses are mediated by different, but identified, receptors. Each hormetic response in this Figure indicates (✹) where the agonist was tested against an antagonist. The (✹) designation also indicates the response (%) for that agonist/antagonist interaction. For a more detailed explanation for the receptor mediated mechanism refer to Supplemental Data 1 for all graphs in Figure 6.

subtypes. Receptor subtype ET_A mediated the stimulatory effect while receptor subtype ET_B mediated the high dose/concentration inhibitory effect (Elferink & De Koster, 1995). In the case of histamine both the low and high dose/concentration effects were reversed by antagonists. The low dose/concentration stimulation was blocked by an H2 antagonist whereas an H1 antagonist blocked the high dose/concentration inhibition (i.e. progesterone production by MA cells; Mondillo et al., 2005; Figure 6.2); this was also the case for testosterone production from testicular macrophages (Khan & Rai, 2007; Figure 6.3).

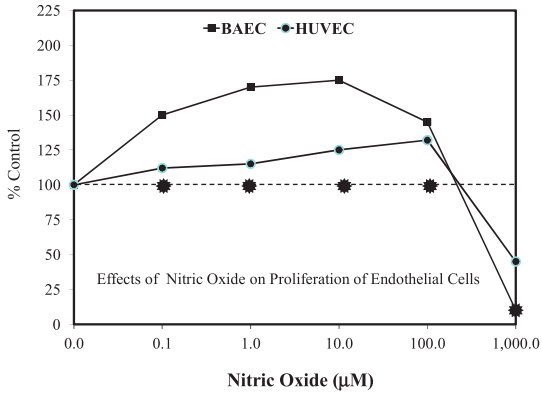
Receptor-based hormetic mechanism #3

Nitric oxide biphasically affected the proliferation of endothelial cells. The low concentration NO-induced proliferation stimulation was blocked by a receptor antagonist (e.g. specific inhibitor of cGC, ODC) but not the high dose/concentration inhibitory response (Isenberg et al., 2005). It was hypothesized that the NO may affect the inhibitory response via the activation of p53 or MKP-1 expression (Figure 7.1).

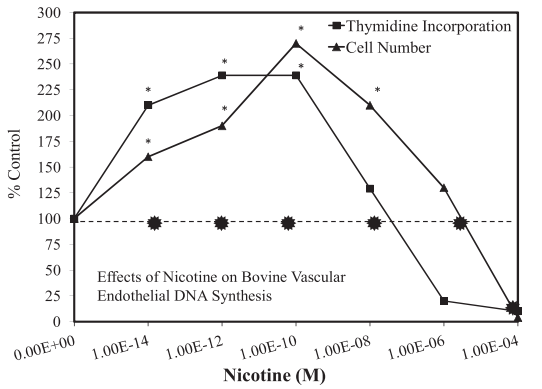
Selected other examples of receptor mediated hormetic responses via mechanism #3

Nicotine has multiple hormetic dose/concentration responses mediated by a receptor-based mechanism (Cucina et al., 2000; Rothem et al., 2009; Villablanca, 1998; Walker et al., 2001). The responses with two osteoblast cell types showed a modest maximum stimulatory response of 25–50% (Rothem et al., 2009; Walker et al., 2001). Two maximum stimulatory responses for endothelial cells were similar, being 150–175% (Villablanca, 1998). The inhibitory responses were a function of how extensive the upper range of doses/concentrations was tested. Several experiments explored a very broad upper dose/concentration range in which the high doses/concentrations were very inhibitory, with responses <10% of the control group values. Receptor antagonists were used for testing against agonist responses in an optimized response concentration within the stimulatory zone. In each case tested, the low dose/concentration stimulatory response was blocked, thus confirming that the stimulatory response was mediated by the nicotine receptor. In two dose/concentration response studies (Rothem et al., 2009; Villablanca, 1998) the receptor

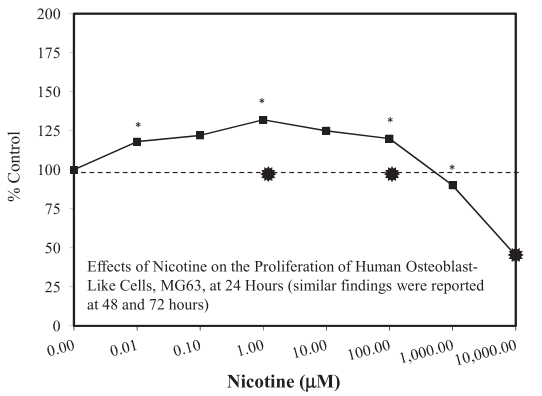
1) Isenberg et al., 2005



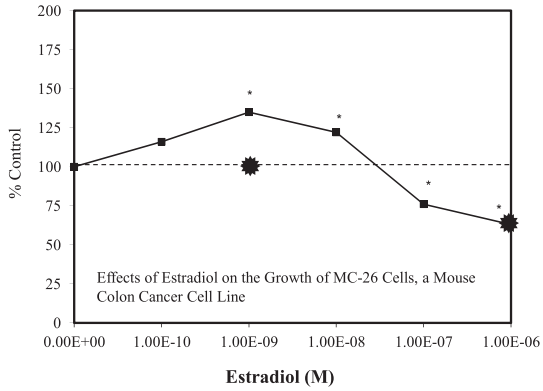
2) Villablanca, 1998



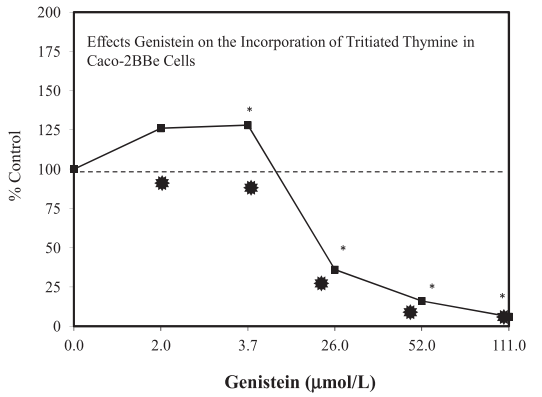
3) Rothem et al., 2009



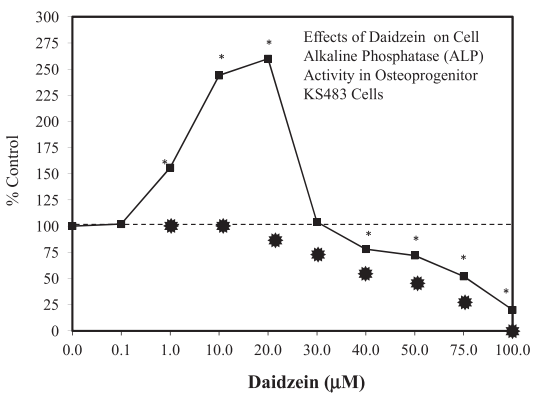
4) Xu and Thomas, 1999



5) Chen and Donovan, 2004



6) Dang and Lowik, 2004



Dang and Lowik, 2004

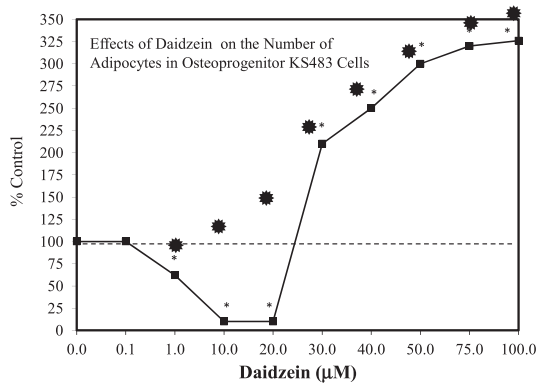
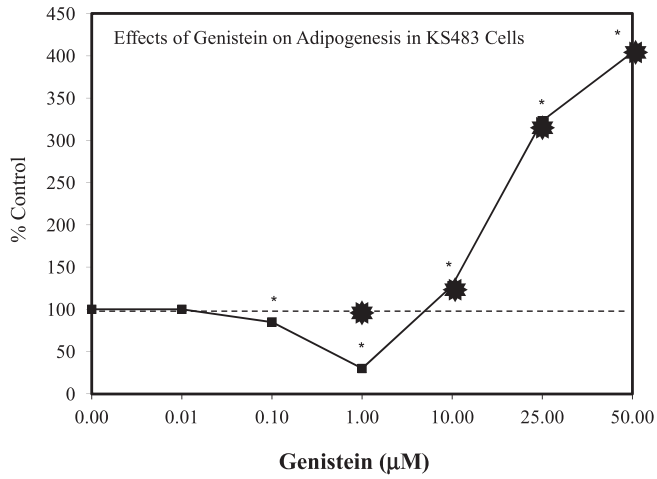
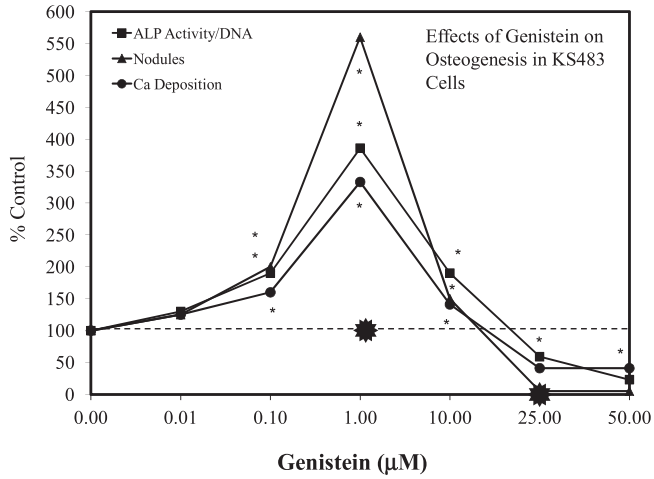


Figure 7. Receptor Mediated Mechanism – Mechanism #3. Examples of hormesis: Dose/concentration response with a receptor mediated mechanism for the low dose/concentration stimulation. However, this receptor did not mediate the inhibitory response. Each hormetic response in this Figure indicates (✷) where the agonist was tested against an antagonist. The (✷) designation also indicates the response (%) for that agonist/antagonist interaction. For a more detailed explanation for the receptor mediated mechanism refer to Supplemental Data 1 for all graphs in Figure 7.

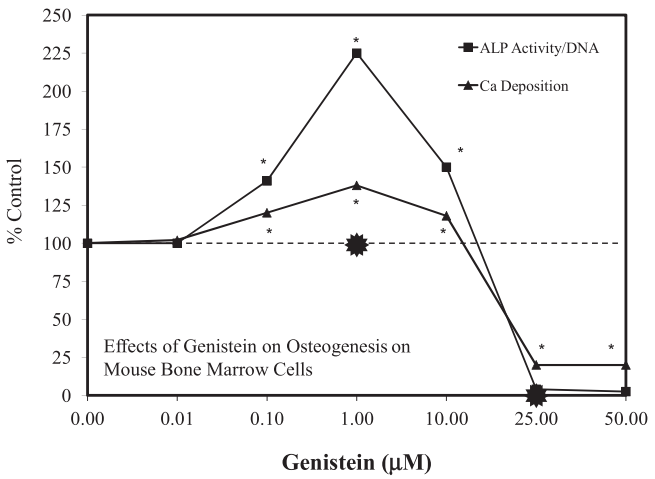
7) Dang et al., 2003



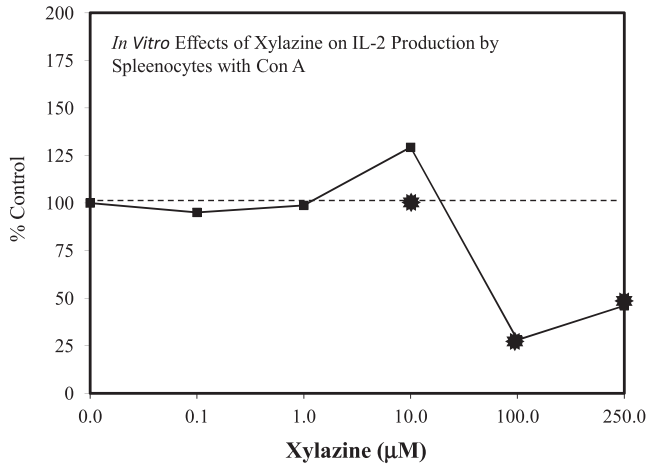
8) Dang et al., 2003



Dang et al., 2003



9) Cupic et al., 2001



10) Schafberg et al., 1997

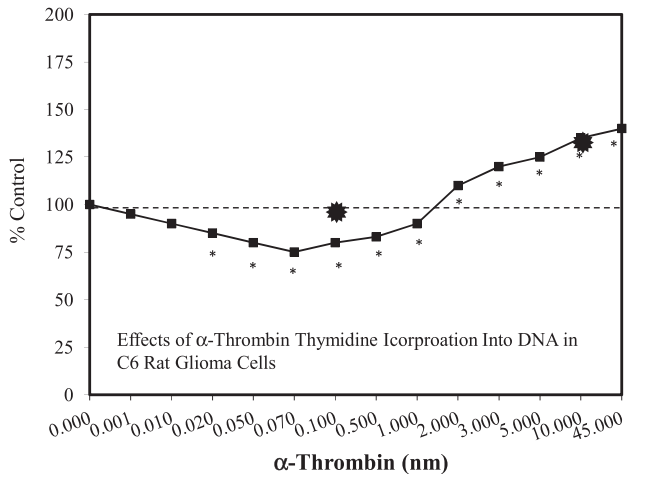


Figure 7. Continued.

agonist was also inhibitory at high dose/concentration. In both of these dose/concentration responses the use of the receptor antagonist failed to reverse the inhibitory effect, indicating that a different mechanism was affecting the high dose/concentration response for these two specific dose/concentration responses (Figures 7.2, 7.3).

Other receptor antagonists blocked the low dose/concentration stimulation but failed to block the high dose/

concentration inhibition induced by the agonist. This is seen with estrogen for MC26 Cells (Figure 7.4; Xu & Thomas, 1995); with genistein in Caco2BBE cells (a colon cancer cell line; Figure 7.5; Chen & Donovan, 2004); for daidzein in KS483 cells (Figure 7.6; Dang & Lowik, 2004); for genistein in KS483 cells (Figures 7.7, 7.8; Dang et al., 2003); and for xylazine in rat spleen cells (Figure 7.9; Cupic et al., 2001); and for α-thrombin with CG rat glioma cells (Figure 7.10; Schafberg et al., 1997).

Antagonist-mediated enhancement of inhibitory responses

In contrast to the above cases of failure of the antagonist to affect the high dose/concentration inhibition, Dang and Lowik (2004; Figure 7.6) demonstrated a situation in which the antagonist enhanced the high dose/concentration inhibitory effect in a consistent fashion for diadzen but not for genistein using the same cellular model (Figure 7.8). Chen and Donovan (2004) also demonstrated a consistent enhancement of the high concentration inhibitory effect with genistein in the CaCa2BBe cell model. Similar mechanistic responses were reported for ouabain (Figure 9A) Bagrov & Fedorova (1998) and xylazine (Figure 9B; Colic et al., 2000).

Cell signaling mediated mechanisms of hormetic dose/concentration responses

Numerous examples of cell signaling based mechanisms for hormetic dose/concentration responses have been reported. Nearly 95% of the approximately 200 examples (Supplemental Data 2) addressed only the low dose/concentration stimulatory mechanism. In the case of receptor-mediated hormetic dose/concentration response mediated mechanisms, approximately 25% of the nearly 200 examples addressed both the low dose/concentration stimulation and high dose/concentration inhibition mechanisms. There is no obvious explanation why this dichotomy exists in mechanism assessment between receptor and cell signaling pathway mediated hormetic dose/concentration responses. Examples of cell signaling pathway mediated hormetic dose/concentration responses for which both stimulatory and inhibitory responses are shown in Figure 8, in accordance with the scheme employed for receptor mediated mechanisms (i.e. mechanism type #'s 1-3 as explained above).

Section summary

These collective findings describe some of the receptor and cell signaling pathway mediated mechanistic complexities underlying hormetic dose/concentration responses and their adaptive strategies for a large number of agents. Biphasic dose/concentration responses can be mediated by a single receptor for both the low dose/concentration stimulatory and high dose/concentration inhibitory effects or both dose/concentration-related effects (stimulatory and inhibitory) can be mediated by different subtypes of the same receptor or may not share the same receptor or a receptor of the same receptor family. Despite these differences in mechanistic strategies the quantifiable features of the dose/concentration responses are similar, suggesting a similar functional and adaptive strategy.

Magnitude of stimulatory response: a comparison of cell signaling phosphorylation versus integrated cellular hormetic dose/concentration responses (e.g. cell proliferation, cell migration)

The hormetic dose/concentration response involves biological endpoints that are highly integrated. These endpoints included cell proliferation, wound healing, immune function, reproductive performance, among others. The hormetic dose/concentration response is characterized by a modest stimulation in the low dose/concentration zone. Yet, these highly integrated responses are dependent upon the activation of cellular receptors and cell signaling cascades.

In the present data set, 51 hormetic dose/concentration responses provided quantitative comparisons of the magnitude of the integrative responses with associated cell signaling phosphorylated proteins such as pERK1/2 and pAKT (Table 5). These cases of integrative cellular responses (e.g. cell proliferation) yielded a median response of 138.5%. In contrast, the cell signaling median response was 224%. On average, the cell signaling response exceeded the integrative responses by about 60%. Two of the signaling responses were unusually high, being 15.0-fold greater than the control in the T47D breast tumor cell line and 11.5-fold greater than the control in the kidney ADPKD genetic disease model (Table 5).

Evaluation of hormetic mechanisms by cell type and endpoint

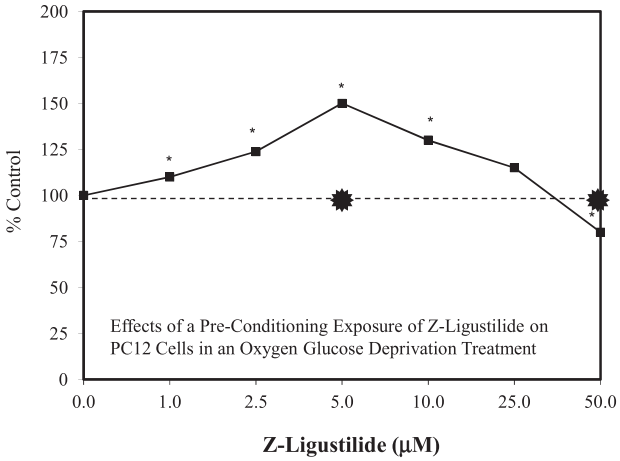
Cell proliferation

Normal cells

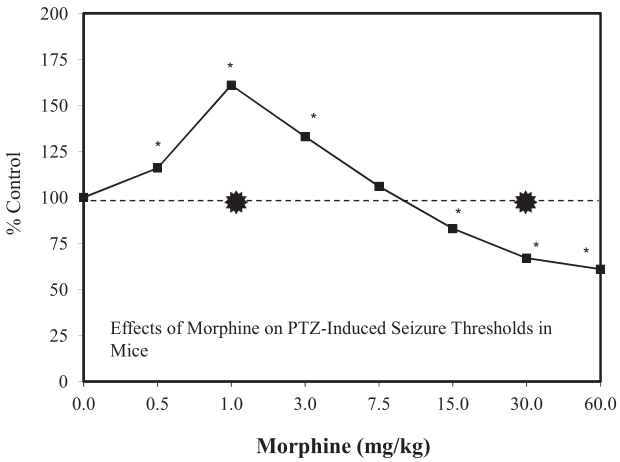
Hormetic mechanisms have been reported in numerous cell types, such as smooth muscle, endothelial, breast, keratocytes, fibroblasts, bone cells, kidney, retinal epithelial cells and others for cell proliferation endpoints in normal (i.e. non-cancer) cells. Within some of the general cell types just listed there are multiple specific types of cells for which hormetic mechanisms were reported. For example, there are six different types of endothelial and smooth muscles cells for which hormetic response mechanisms are presented. The mechanisms were assessed via receptor antagonists and/or cell signaling pathway inhibitors. Despite the wide range of study hypotheses, methods and research strategies, several consistent patterns have emerged with respect to hormetic mechanisms and cell signaling pathways for the cell proliferation endpoint. There is a consistency in pathway mediated hormetic responses within a general cell type (e.g. endothelial cells), independent of the agonist. Second, there is a similar, but not as consistent pattern of mechanism mediated pathways across the broad range of cell types. That is, most cell types utilize the MAPK/ERK1/2 cell signaling pathway to affect the hormetic dose response for cell proliferation, revealing a general mechanistic consistency. A cell type not involving the ERK1/2 for cell proliferation was the V79 Chinese hamster lung fibroblast cell which utilized the MAPK p38 pathway (Kim et al., 2001). The ERK1/2 pathway can display

Mechanism #1

1) Qi et al., 2012

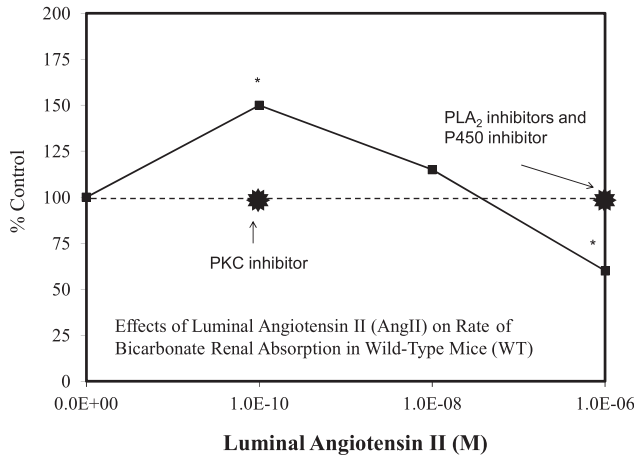


2) Homayoun et al., 2002

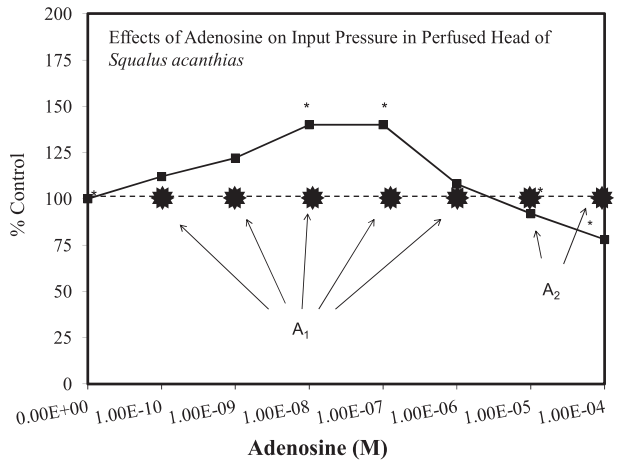


Mechanism #2

1) Zheng et al., 2003



2) Pellegrino et al., 2005



3) Jiang et al., 2009

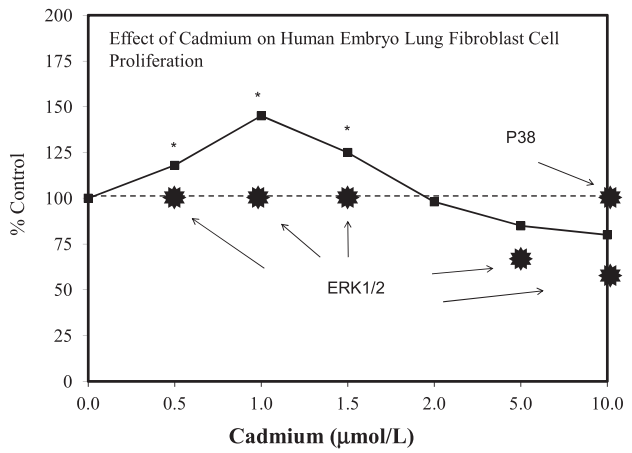
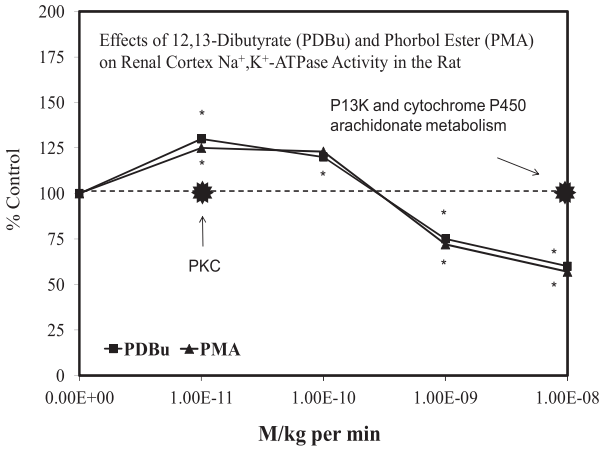


Figure 8. Examples of cell signaling mechanisms of hormetic dose/concentration responses. Each hormetic response in this Figure indicates (✹) where the agonist was tested against a cell signaling pathway inhibitor. The (✹) designation also indicates the response (%) for that agonist/antagonist interaction. For a more detailed explanation for the receptor mediated mechanism refer to Supplemental Data 2 for all graphs in Figure 8.

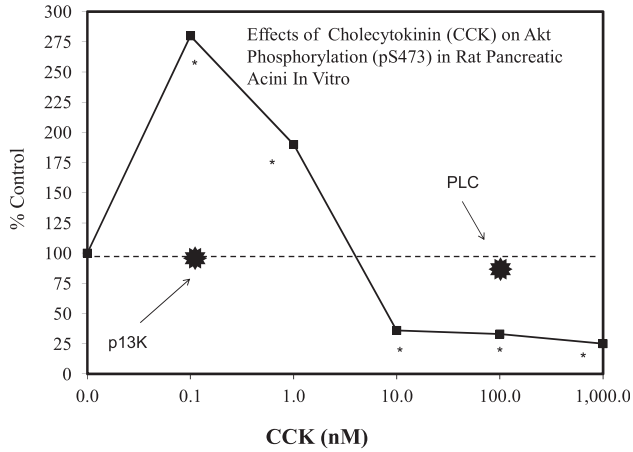
differential linkage with other cell signaling pathways, depending on the cell type. Several cell types, including intestine (Mantha & Jumarie, 2010) and endothelial (Kung et al., 2008) employ an ERK1/2-p38/and/or JNK linkage to affect hormetic dose responses. In the case of Mantha & Jumarie (2010), ERK1/2-p38 was involved in mediating the hormetic response for cell proliferation of intestine cells, with JNK being absent. In contrast to the intestine cell model used

Mechanism #2

4) Beltowski et al., 2004

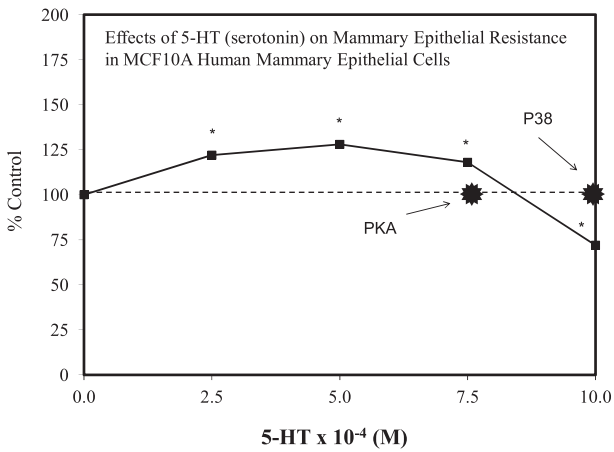


7) Berna et al., 2009

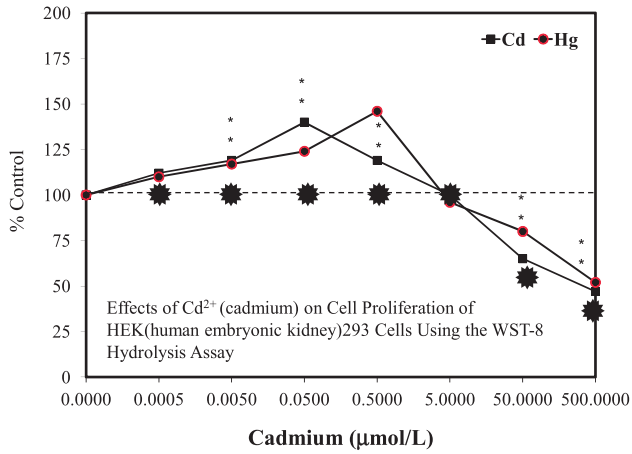


Mechanism #3

5) Pai and Horseman, 2008



1) Hao et al., 2009



6) Lelievre et al., 1998

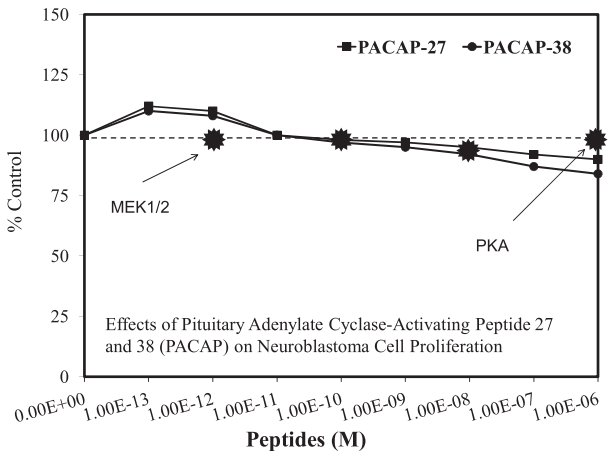
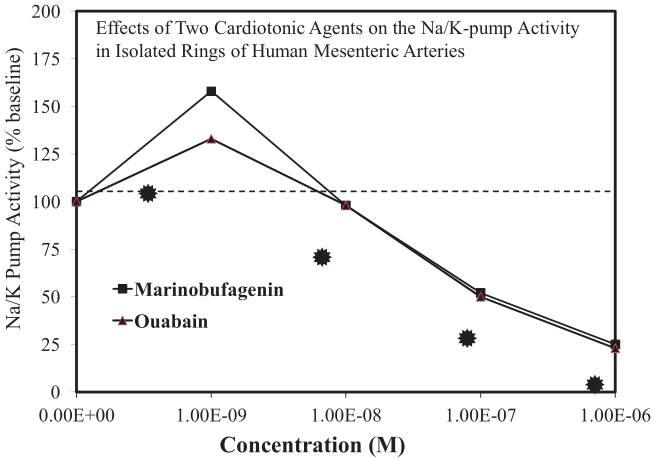


Figure 8. Continued.

(A) Bagrov and Fedorova, 1998



(B) Colic et al., 2000

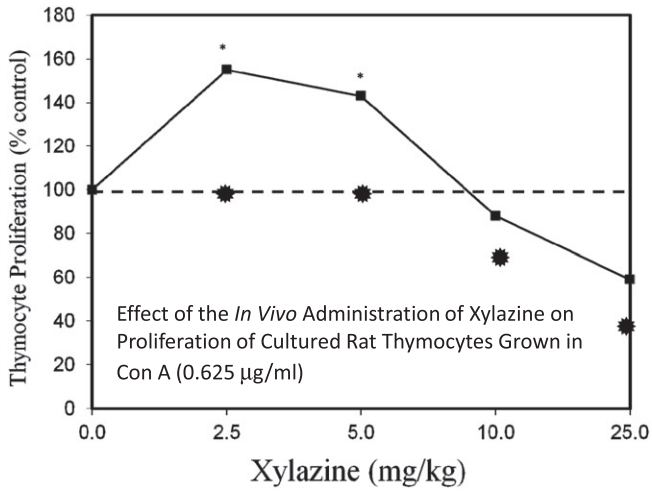


Figure 9. Examples of hormesis where the antagonist for the receptor blocked the low dose/concentration stimulation but enhanced the inhibitory response. Each hormetic response in this Figure indicates (✷) where the agonist was tested against an antagonist. The (✷) designation also indicates the response (%) for that agonist/antagonist interaction.

by Mantha & Jumarie (2010), human embryonic lung and kidney cells displayed an ERK1/2-JNK pathway linkage with p38 involvement being absent (Hao et al., 2009). These findings reveal a common general cell signaling pathway mediating the hormetic cell proliferation response in a wide range of normal cell types.

Tumor cells

Hormetic mechanisms were demonstrated in 14 different tumor cells affecting a broad range of tumor types, including pancreatic, prostate, colon, esophageal, testicular, breast, neural and leukemia. In the majority of cases the receptor that mediated the low dose cell proliferation stimulation response was identified, including estrogen, CCK, glucocorticoid, PKC and PKA. In contrast to research with normal cells, there were fewer examples of studies published on hormetic responses and cell signaling pathways in tumor cell lines even though there is a substantial literature on hormetic responses for a very broad range of tumor cells (Calabrese, 2005a). In several cases investigators identified an essential cell signaling pathway as well as the receptor (e.g. genistein exposure activated the estrogen receptor and the PI3K pathway within a prostate tumor cell model (PC3 cells; El Touny & Banerjee, 2009); hydrogen peroxide activated PKC and ERK1/2 within a prostate tumor cell model (Du-145 cells; Wartenberg et al., 1999). While MAPK-ERK1/2 was essential for peptide induced neuroblastoma (Lelievre et al., 1998), it had no involvement with the effects of BHA on hormetic responses in testicular cells (Bouskine et al., 2009). In contrast to the capacity to derive some measure of general hormetic cell signaling mechanism for normal cells and cell proliferation, the findings are too limited at present to do so for tumor cells.

Cell migration

Fourteen cell types displayed hormetic mechanisms for cell migration. These cells represent a wide range of cell types, including various tumor cell lines, immune cells, nerve, intestinal, kidney and retinal cells from multiple species. Of the numerous mechanism studies for cell migration endpoints, mechanisms were only clarified for low dose/concentration stimulatory responses.

The mechanistic evaluation for cell migration included an assessment of specific receptors or specific cell signaling pathways. Multiple receptors (e.g. TGF-B, EGF, PDCF, PP-y and H4) were shown to mediate the low concentration stimulatory responses. Cell signaling pathways were observed to mediate the low concentration cell migration stimulatory responses. For example, cell signaling pathways mediated cell migration stimulatory responses with JNK for OVCAR-3 cells (Cheung et al., 2006), P13K/AKT for HUVEC (Kook et al., 2003) and p38 for rat aortic smooth muscle (Zhou et al., 2009).

The quantitative features of the hormetic response for cell migration typically displays stimulatory responses several fold greater than the typical hormetic dose response (Calabrese, 2005b). This was also the case in the examples reported in the present article. Whether these differences in the amplitude of the stimulation are unique to the cell types, endpoints or the experimental methodology remains to be clarified.

In contrast to findings with non-cancer cells for the cell proliferation endpoint, involvement of ERK1/2 as mediating the hormetic dose response was not a common feature of cell migration. This was observed with the rat aortic smooth muscle (Zhou et al., 2009), ovarian tumor cells (Cheung et al., 2006), eosinophils (Kobayashi et al., 2005), microglia (Bianchi et al., 2011) as well as RPE (Hollborn et al., 2010).

Other endpoints

Numerous hormetic dose responses have endpoints other than cell proliferation and cell migration which are mediated by receptor and/or cell signaling pathways. Table 6 provides examples in which specific hormetic dose responses are mediated by pERK1/2, p38, p13K, and their interactions.

Table 6. Selected hormetic mechanisms for multiple endpoints.

	Reference
ERK1/2 Pathway Mediated Hormetic Response	
Estrogen-induced leptin expression on placental cells	Gambino et al., 2010
Peroxynitrite-induced O2 generation from human PMN	Lee et al., 2000
HMGb1-induced viability of 3T3 cells	Ranzato et al., 2010
p38 Pathway Mediated Hormetic Response	
Fluvastin-induced angiogenesis with endothelial and fibroblast co-cultures	Uruno et al., 2008
Vitamin D3 effect on p38 MAPKinase phosphorylation in intestinal cells in male adults Wistar rats	Pardo et al., 2006
Stroke Models	
Methamphetamine neuroprotection-rat hippocampus – P13K/Akt	Rau et al., 2011
Z-ligustilide cell survival-PC12 cells – P13K/Akt	Qi et al., 2012
Multiple Pathway Compounds	
HX/XO on DNA synthesis of Rat T-1 cells – ERK1/2, P12K and mTOR	Duleba et al., 2004
Jocoba Liquid Wax – scratch wound tests – mTOR & P13K; ERK1/2 and p38 involved to lesser degree	Ranzato et al., 2011
Stimulatory and Inhibitory Pathways	
5-HT effect on mammary epithelial resistance in MCF 10 cells – PKC mediated stimulation; p38 mediated inhibition	Pai and Horseman, 2008
CCK induced Akt phosphorylation in rat pancreatic acini – P13K mediated stimulation; PLC mediated inhibition	Berna et al., 2009
Other Pathways	
Morphine – PTZ-seizure – NO	Homayoun et al., 2002
TNF – transcient electronically stimulated cardiomyocytes – PLA2	Amadou et al., 2002
Ethanolamine – protect cardiac tissue from damage – JAK-STAT-3	Kelly et al., 2010
Other Endpoints	
Resveratrol – HO-1 promotor activity aortic smooth muscle (human): Receptor-NF-kB; Pathway-I$\kappa\beta\alpha$ a phosphorylation (ERK1/2, JNK, & p38 – shown not to be involved)	Juan et al., 2005
Vitamin D3 – effect on p38 MAKinase; Pathways-phosphorylation in intestinal cells of male adults Wistar rats; p38 a/b blocked (SB203530 inhibitor); PKA cSrc inhibitor blocked p38 phosphorylation	Pardo et al., 2006
PDBu/Phorbol ester – effect on renal cortex Na+K+-ATpase in the rat – PKC	Beltowski et al., 2004
Platelet lysate – effect on cell viability in C2Cl2 mouse myoblasts – p38 and P13K pathways needed; ERK1/2 shown not to be involved	Ranzato et al., 2009b
LPS – wound repair on human primary muco carcinoma cells – tyrosine kinase phosphorlyation	Koff et al., 2006

It also provides information on situations in which there is an absence of pathway involvement.

Dose–response assessment

The upper end of the dose response relationship has been differentially assessed for hormetic dose responses, depending on the number of concentrations tested, their spacing and whether receptor/cell signaling pathways were evaluated. Many dose response relationships display steep inhibitory responses such as with 5-HT (Pakala & Benedict, 1998; Figure 10). This specific dose response also assessed the concentration response below the initiation of the stimulatory response, that is, the entire dose response continuum. Other investigators have broadly assessed the response continuum (Dang & Lowik, 2004; Puzzo et al., 2008; Wang et al., 2005, 2011). In contrast, the study of Hsu et al. (1999) characterized the upper end of the dose response in a comprehensive fashion while having four concentrations in the low dose stimulatory zone with two concentrations being assessed for receptor mediation by an antagonist. Despite this extensive effort by Hsu et al. (1999) it is not known what the extent of the stimulatory range is (Figure 11).

In-vivo mechanism research

While the strong majority of the hormetic mechanism studies are performed within an *in vitro* system, some experiments employed whole animals (Boccia et al., 1998; Duranski et al., 2005; Homayoun et al., 2002; Houshmand et al., 2009; Kawabata et al., 1994; Kelly et al., 2010; Kim et al., 2008; Ogura et al., 2009; Rogers & Eastell, 2011). For example, Houshmand et al. (2009) showed that a preconditioning treatment of oxytocin protected against IR induced myocardial damage in male Sprague-Dawley rats. The protective effect of the oxytocin was blocked by an oxytocin antagonist (Figure 12). A similar finding was reported by Kelly et al. (2010) that pretreatment with ethanelamine decreased infarct size in an hormetic fashion (Figure 13). The protection disappeared in cardiomyoctes when using STAT-3 deficient mice and in mice treated with a JAK-STAT-3 inhibitor. Kawabata et al. (1994) reported that L-arginine induced a biphasic nociception dose response in male mice. The administration of the NOS inhibitor L-NAME blocked both the stimulatory and inhibitory responses (Figure 14).

Discussion

This article represents the first extensive documentation of hormetic dose/concentration response mechanisms. The strategy was developed to document hormetic stimulatory responses that were mediated via receptor and/or cell signaling pathways and to require that the stimulatory responses be blocked by the use of either receptor antagonists or specific cell signaling pathway blockers. Evidence is presented of several hundred hormetic dose/concentration

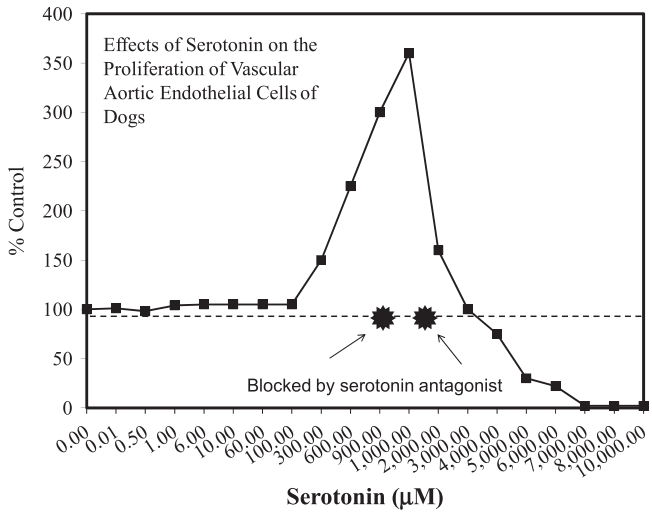


Figure 10. Effects of serotonin on the proliferation of vascular aortic endothelial cells of dogs (*Source*: Pakala & Benedict, 1998).

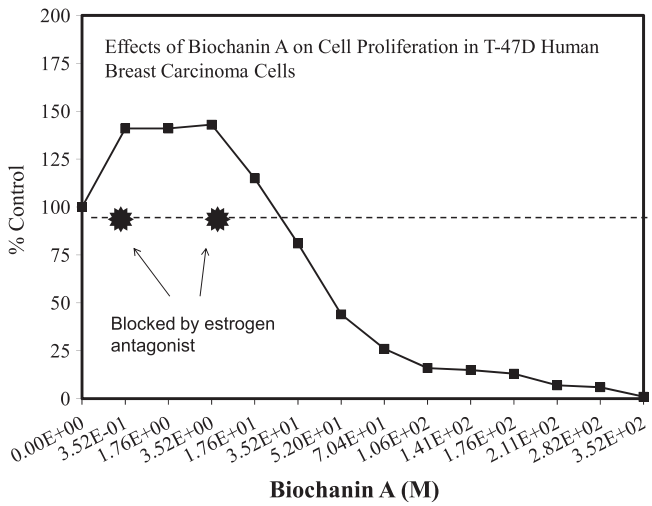


Figure 11. Effects of biochanin A on cell proliferation in T-47D human breast carcinoma cells (*Source*: Hsu et al., 1999).

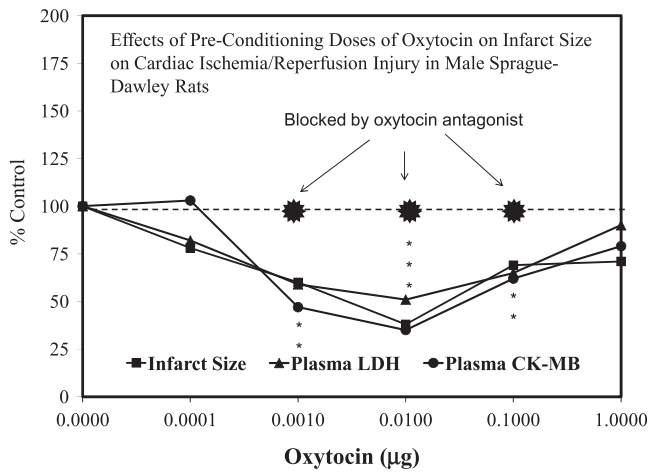


Figure 12. Effects of pre-conditioning doses of oxytocin on infarct size on cardiac ischemia/reperfusion injury in male Sprague-Dawley rats (*Source:* Houshmand et al., 2009).

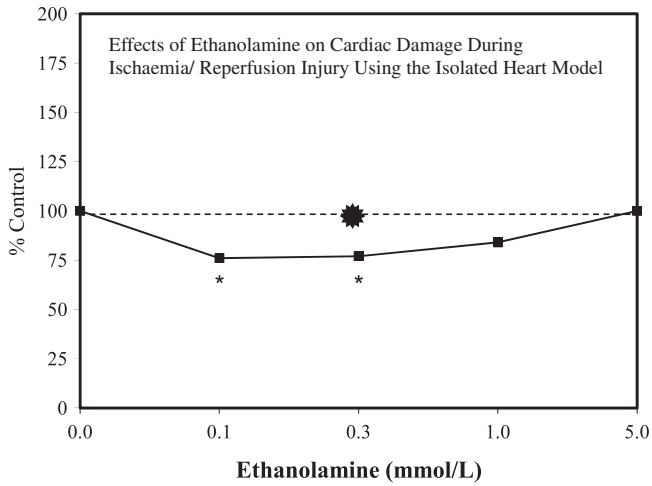


Figure 13. Effects of ethanolamine on cardiac damage during ischaemia/ reperfusion injury (*Source*: Kelly et al., 2010).

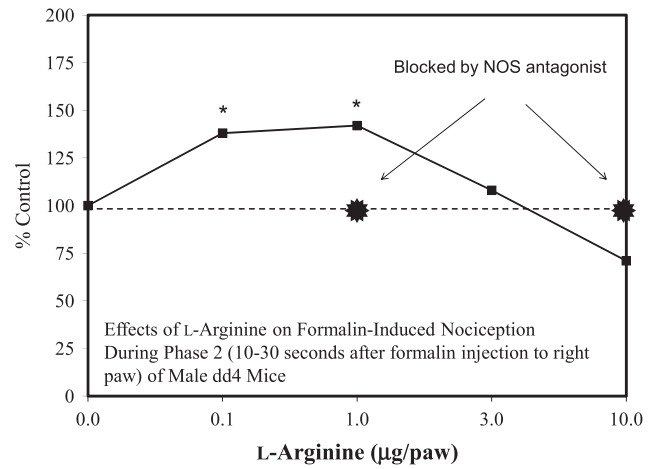


Figure 14. Effects of L-arginine on formalin-induced nociception during phase 2 (10–30 seconds after formalin injection to right paw) of male dd4 mice (*Source*: Kawabata et al., 1994).

responses for which the molecular mechanism has been demonstrated as being mediated via a specific receptor or one or more cell signaling pathways. The range of biological models, endpoints and inducing agents for which mechanisms have been demonstrated is quite broad, indicating that the findings are generalizable.

Of particular interest is that considerable evidence demonstrated that cells showing a hormetic dose response for cell proliferation mediate this via the MAPK-ERK1/2 cell signaling pathway. This was the case for 14 different cell types, with the only exception being V79 cells in which p38 was essential whereas ERK1/2 involvement was absent. In contrast, when similar cell types display hormetic dose responses for cell migration, the hormetic response is typically mediated via a different cell signaling pathway such as p38 (e.g. rat aortic smooth muscle) and/or JNK [ovarian tumor cell lines (Cheung et al., 2006), and microglia (Bianchi et al., 2011)].

The experiments included in this assessment appear to be representative of the large body of hormetic

dose/concentration responses in multiple databases (Calabrese & Blain, 2005, 2009, 2011). For example, the numerous studies comprising the multiple hormetic dose/concentration response databases revealed that the maximum response is commonly in the 30–60% range greater than the control is similar to that reported here for both receptor and cell signaling mediated mechanisms.

The overall findings indicate that the quantitative features of the hormetic dose/concentration response are independent of mechanism, regardless of which cell type receptor or cell signaling pathways are involved, and their possible interactions. This observation is significant since it indicates the maximum therapeutic and/or undesirable effects that could be induced by a chemical, drug or ionizing radiation in the below threshold zone. This observation also suggests that the high consistency of the maximum stimulation of hormetic dose/concentration responses may be a measure of the plasticity across biological systems and different levels of biological organization (cell, organ, and organism; Calabrese & Mattson, 2011).

Mechanistic applications

Within this section five examples of mechanism application are briefly summarized. They deal with 1) the concept of developmental hormetic mechanisms, 2) a comparison between normal and diabetic animal models and hormetic responses, 3) how the same agonist acts via differing receptor subgroups to produce the same or opposing effects, 4) the concept of a molecular switching mechanism that accounts for the occurrence of the low dose/concentration stimulating processes in human breast cancer cells and 5) how the same agonist produces two different types of hormetic effects (i.e. cell proliferation and cell migration).

Developmental hormetic mechanisms

The same agent (e.g. cadmium) induced low dose/concentration stimulation in two different but closely related cell types (human embryonic kidney cells and human embryonic lung fibroblasts) via the ERK1/2 pathway and in the same optimal stimulatory dose/concentration range (see Supplemental Data 2, figures on pages 38 and 54; Hao et al., 2009; Jiang et al., 2009). The JNK pathway was also involved in the low dose/concentration stimulatory responses in both cell types. The ERK1/2 pathway was not implicated in the high dose/concentration related inhibitory responses. While p38 pathway inhibitors had no effect on the high dose/concentration inhibitory response in the human embryonic kidney cells, these p38 inhibitors blocked the inhibitory response of the human embryonic lung fibroblast cells, a finding that suggests a differential developmental pattern that mediates the mechanistic diversity of the hormetic-biphasic dose/concentration responses in such human embryonic cells.

High risk groups and hormetic mechanism: Normal versus diabetic

The mechanistic assessment revealed a differential response pattern between normal and diabetic (STZ-induced) rats (see Supplemental Data 1, Figure on page14; Kuntz et al., 2002). In the case of non-diabetic male Wistar rats, cholecystokinin octapeptide (CCK-8) biphasically affected DNA synthesis in cultured pancreatic acini. The low dose/concentration stimulation was blocked by the CCK-8 antagonist L364,718. In contrast to the non-diabetic rat, diabetic (STZ-induced) rats displayed only a dose/concentration-dependent increase with no biphasic response. Follow up experiments revealed that the monotonic dose/concentration response in the diabetic rat was mediated by a single class of low affinity CCK_1 receptors. In contrast, there were two classes of CCK-8 binding sites (high and low affinity) in non-diabetic rats. These findings demonstrate a mechanistic process by which an hormetic dose/concentration response can be mediated or lost.

Agonist activity via differing receptor subgroups

The endogenous agonist histamine affects a number of biphasic dose/concentration response relationships via the use of two receptor subtypes (H1 and H2). In general, findings revealed that the low dose/concentration stimulatory response was blocked by the H2 antagonist for the production of testosterone (see Supplemental Data 1, figure on page 31; Khan & Rai, 2007) and progesterone (see Supplemental Data 1, figures on pages 34 and 35; Mondillo et al., 2005) and fibroblast proliferation during normal wound healing (see Supplemental Data 1, figure on page 32; Kupietzky & Levi-Schaffer, 1996). H2 antagonists had no effect on the high dose/concentration inhibitory responses. In contrast, the H1 antagonists act in the reverse manner, blocking the high dose/concentration inhibition while having no effect on the low dose/concentration stimulation. A variation in this receptor mediated mechanism pattern was reported by Leonardi et al. (1999; see Supplemental Data 1, figure on page 33) in which both H1 and H2 agonists blocked histamine induced VKC-derived fibroblast proliferation. However, only the H2 antagonists blocked VKC-fibroblast migration. The observation that two receptor subtypes, using the same agonist, mediate the same stimulatory response is important. This indicates that despite the presence of an additional activity the quantitative features of the hormetic dose/concentration response remain the same.

Hormesis via a molecular switching mechanism

The molecular mechanism for an β-estradiol ($E_2\beta$)-induced biphasic dose/concentration-response for ER (estrogen receptor)-negative cancer cell models (MDA-MB-231, MDA-MB-436) was evaluated by Zhang et al. (2012; see Supplemental Data 2, figure on page 63). The ER-negative breast cancer cells displayed a modified ER when compared to ER-positive breast cancer cells. That is, the estrogen receptor in the ER-negative breast cancer cells is expressed on the cell surface and lacks both AF1 and AF2 domains. Zhang et al. (2012) discovered the presence of an on/off switch for ER activation. The molecular switching process involved the phosphorylation of a specific amino acid (Src-YS27) of the modified ER, a process that inactivates Src. Of considerable importance is that the $E_2\beta$ at 1 nM but not at 5 μM, phosphorylated MAPK/ERK, thereby modulating the Src/EGFR/STAT5 pathways/activity. Knockdown of the ER

variant prevents the biphasic estrogen signal in the MDA-MB-231/MDA-MB-436 ER-negative breast cancer cells. Thus, in these ER-negative cell models Src functions as a molecular switch for the biphasic estrogen signal which is concentration-dependent.

With the exception of the paper by Zhang et al. (2012) hormetic biological switching mechanisms were not addressed in the papers cited here. However, the collective data suggest the existence of several possible hormetic biological switching mechanisms, based on observations that hormetic responses can be mediated by a single receptor, $\geq$2 receptor subtypes, $\geq$2 different receptors, and a receptor–non-receptor interaction mediated mechanism. For example, cases where the low dose stimulation and the high dose inhibition are mediated by the same receptor reveals an auto-regulatory switch function. Examples that support this interpretation follow hormesis receptor mechanism #1 (Figure 3A). In other cases the interaction of two receptor sub-types mediates the biphasic dose/concentration response and follow hormesis receptor mechanisms 2 and 3 (Figures 3B and 3C). Similar biphasic dose/concentration responses that are mediated by the occupancy of a single receptor have been reported (Quirk & Funder, 1988; Quirk et al., 1986a, 1998b) for highly specific glucocorticoids. These authors suggested the existence of both turn on and turn off acceptor sites in the nucleus that could serve as a switching device for gene activation. Each of these types of mechanisms operate within a regulatory context with likely concentration-dependent switching processes, some of which maybe mediated by enzymatic tags involving reversible protein phosphorylation (Buchwald et al., 2001). Recent further support for the concept that hormetic dose response signaling mechanisms are dependent on different agonist concentration gradients (Calabrese & Baldwin, 2001c) have been reported by Nalesso et al. (2011). These researchers found that the Wnt signaling pathway is dependent on the concentration gradients of Wnt ligands which affect critical tissue patterning during embryogenesis (Kestler & Kühl, 2011).

Cell proliferation versus cell migration

There is a growing general recognition that cell proliferation requires ERK1/2 activation (Sharma et al., 2003). This has been demonstrated in the present article with numerous agonists with a broad range of cell types [e.g. LPA – aorta vascular smooth muscle of the NZ White rabbit (Faustino et al., 2007); epinephrine – mouse embryonic stem cells (Kim et al., 2008); curcumin – mouse multipotent neural progenitor cells (Kim et al., 2008); ionizing radiation – rat mesenchymal stem cells (Liang et al., 2011); neoeriocitrin – osteoblastic MC3T3-C1 cells (Li et al., 2011); cadmium – CaCo2 cells (Mantha & Jamarie, 2010); DHEA – bovine aortic endothelial cells (Liu et al., 2008)]. Cell migration has commonly been reported to be dependent of the activation of p38 (Hedges et al., 1999; Iijima et al., 2002; Matsumoto et al., 1999; Tangkijvanich et al., 2002) [e.g. HMGB1 – 3T3 cells (Ranzato et al., 2010); PL – C2CL2 mouse myoblast (Ranzato et al., 2009b); LPA – rat arotic smooth muscle cells (Zhou et al., 2009)]. Sharma et al. (2003) proposed a model by which the same event (e.g. injury) leads to the activation of two parallel signaling pathways with one leading to cell proliferation and the other to cell migration. The two pathways integratively interact to achieve an optimized wound healing response (Figure 15), a prediction that was observed in a number of examples of the present article as noted above.

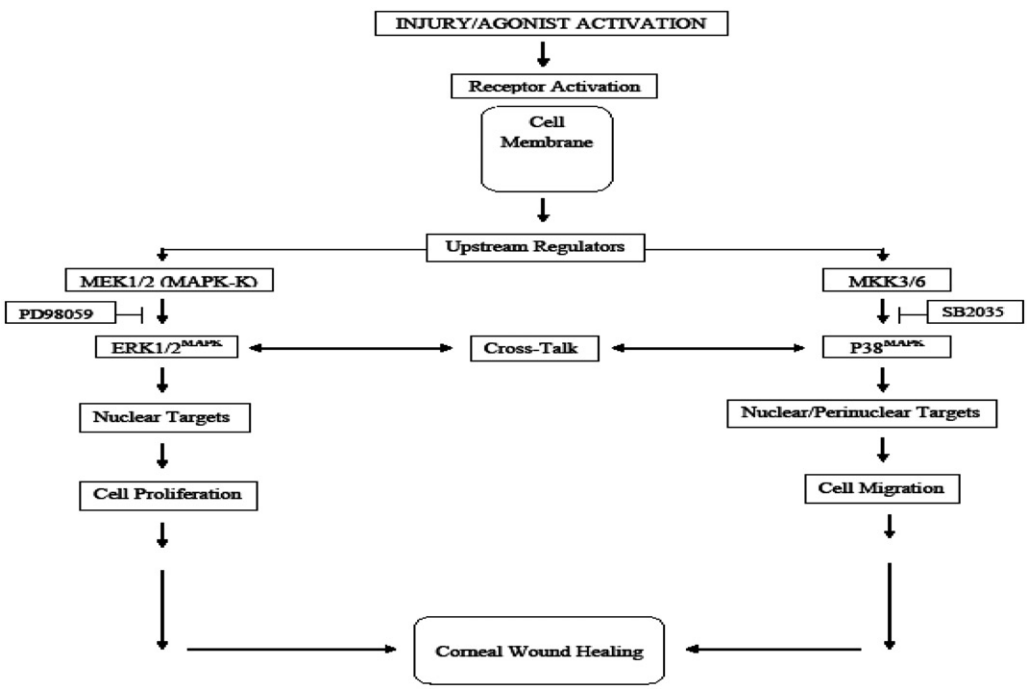


Figure 15. Schematic representation of activation of corneal epithelial wound healing by MAPK. Injury induces the release of growth factors (HGF and KGF) that activate their receptors and induce the coordinated activation and cross-talk between ERK1/2 and p38. Blockade of either signaling cascade at the level of MEK (MAPK-K) or p38 activates the other kinase. These two pathways act together to achieve optimal healing response. Upstream regulation of these kinases has not been defined in this model (*Source:* Sharma et al., 2003).

The interaction of these pathways can be complex and different across cell types (Sharma et al., 2003). For example, blocking the ERK1/2 pathway can trigger the up-regulation of p38. The reverse situation can also be the case, showing communication between the MAP kinases. Various permutations of such receptor cross talk may exist in different cell types, such as one-way cross talk, inhibitory cross talk and other possibilities. Thus, even though there is the general scheme for pathways mediating specific endpoints such as cell proliferation, cell migration and wound healing, there can be considerable variation in their dose optimality and temporal expression.

Final perspectives

The documentation and assessment of the current extensive set of hormetic mechanisms represents a significant and continuing progression to better understand hormetic dose/concentration responses and their biomedical and toxicology significance. Approximately 20 years ago it was uncertain whether hormetic dose/concentration responses were reproducible or merely an occasional paradoxical dose/concentration response of unknown biological significance. However, it has now been extensively and reproducibly shown that hormetic dose/concentration responses are common and broadly generalizable, being independent of biological model, endpoint, inducing agent, and level of biological organization and with broad clinical application. Of further significance is that the hormetic dose/concentration response was also found to better predict responses in the low dose/concentration zone than with the threshold or LNT models in multiple, independent and extensive databases using a priori entry and evaluative criteria (Calabrese & Baldwin, 2001b; Calabrese & Blain, 2011). The current article addresses the long standing need to better demonstrate the mechanistic foundations of hormetic dose/concentration responses and to integrate these findings within the framework of molecular biology. Nonetheless, despite the considerable advances in the clarification of receptor and/or cell signaling mediating pathways mediated hormetic mechanisms, additional focused mechanism research is needed to account for the quantitative features of the hormetic dose responses and how these features could be modulated within a biological switching context that is dose/concentration dependent.

Declaration of interest

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Critical Review

HORMESIS: WHY IT IS IMPORTANT TO TOXICOLOGY AND TOXICOLOGISTS

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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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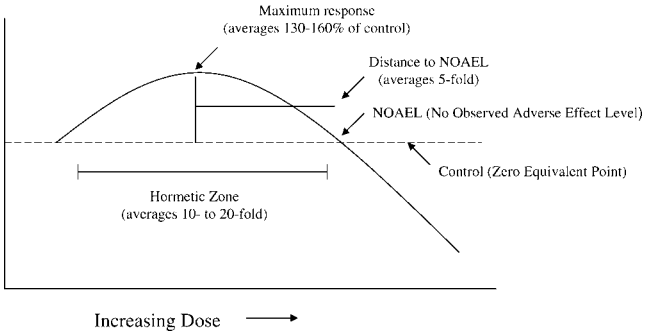


Fig. 1. Dose–response curve depicting the quantitative feature of hormesis [10].

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.

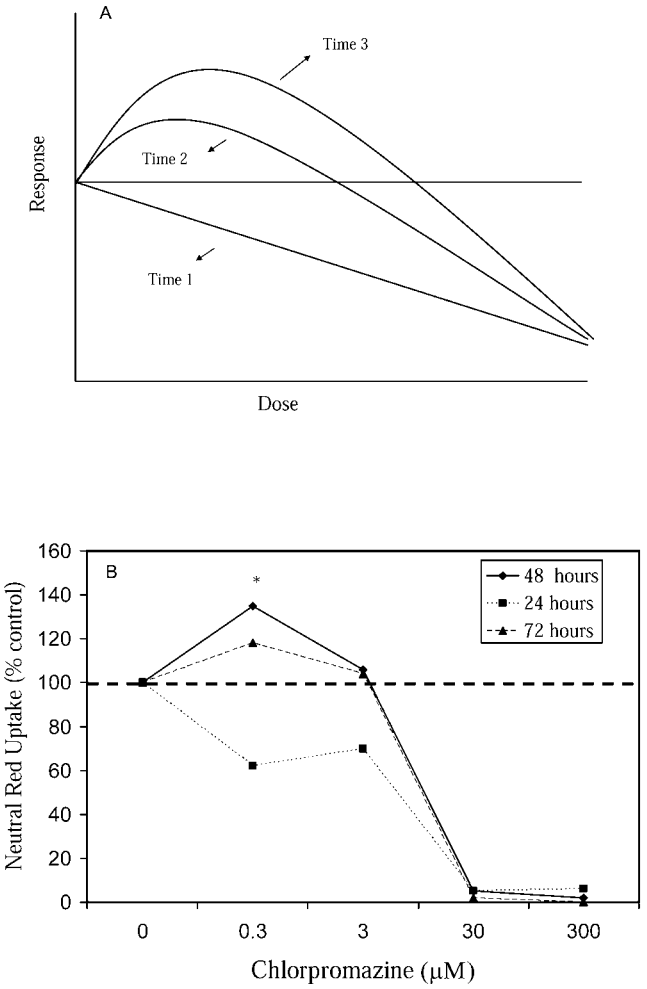


Fig. 2. Hormetic dose–time–response relationship: Modest overcompensation following a disruption in homeostasis (for review, see [8,167]).

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

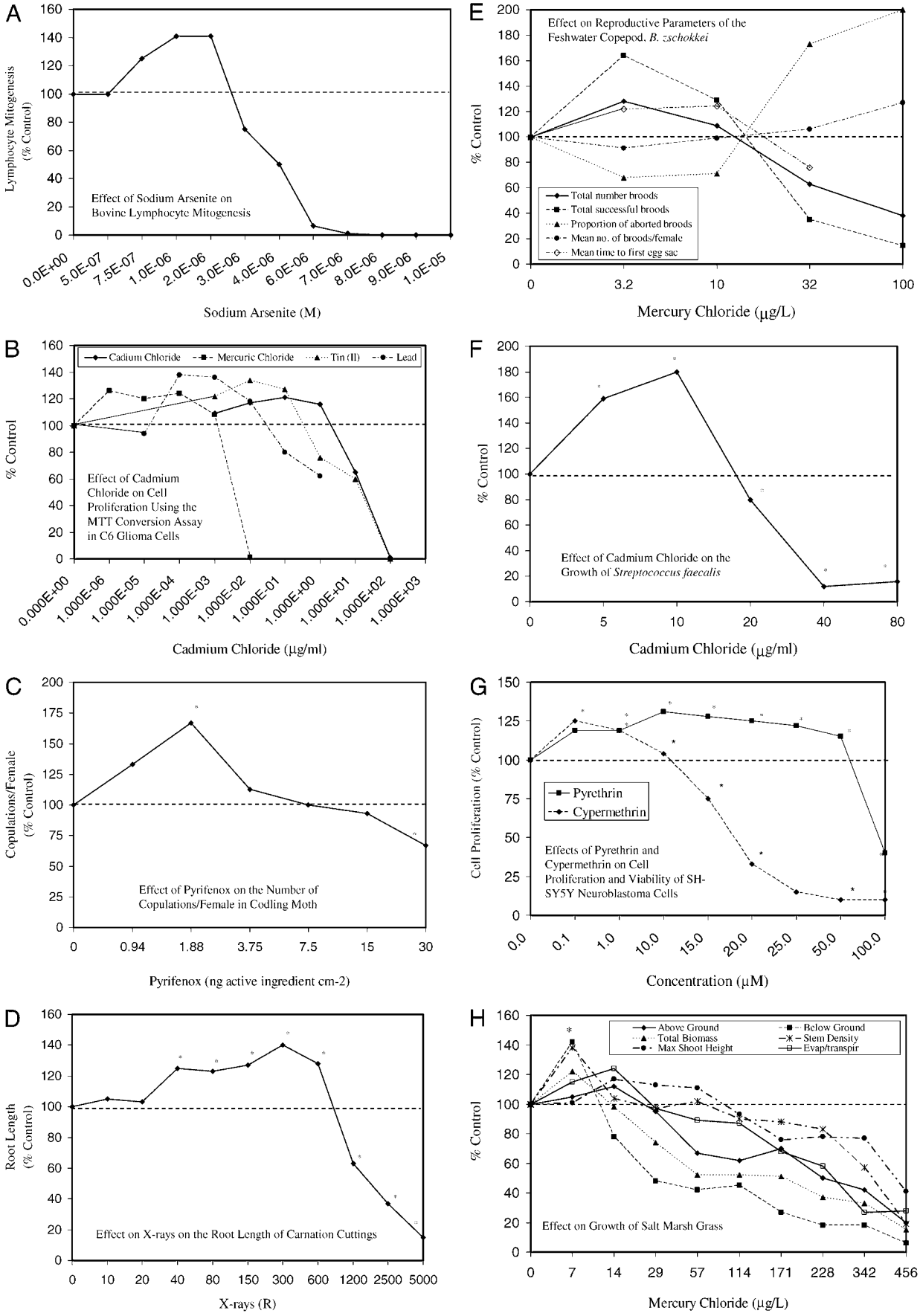


Fig. 3. Multiple hormetic responses [168–184]. Asterisks denote statistical significance ($p \leq 0.05$).

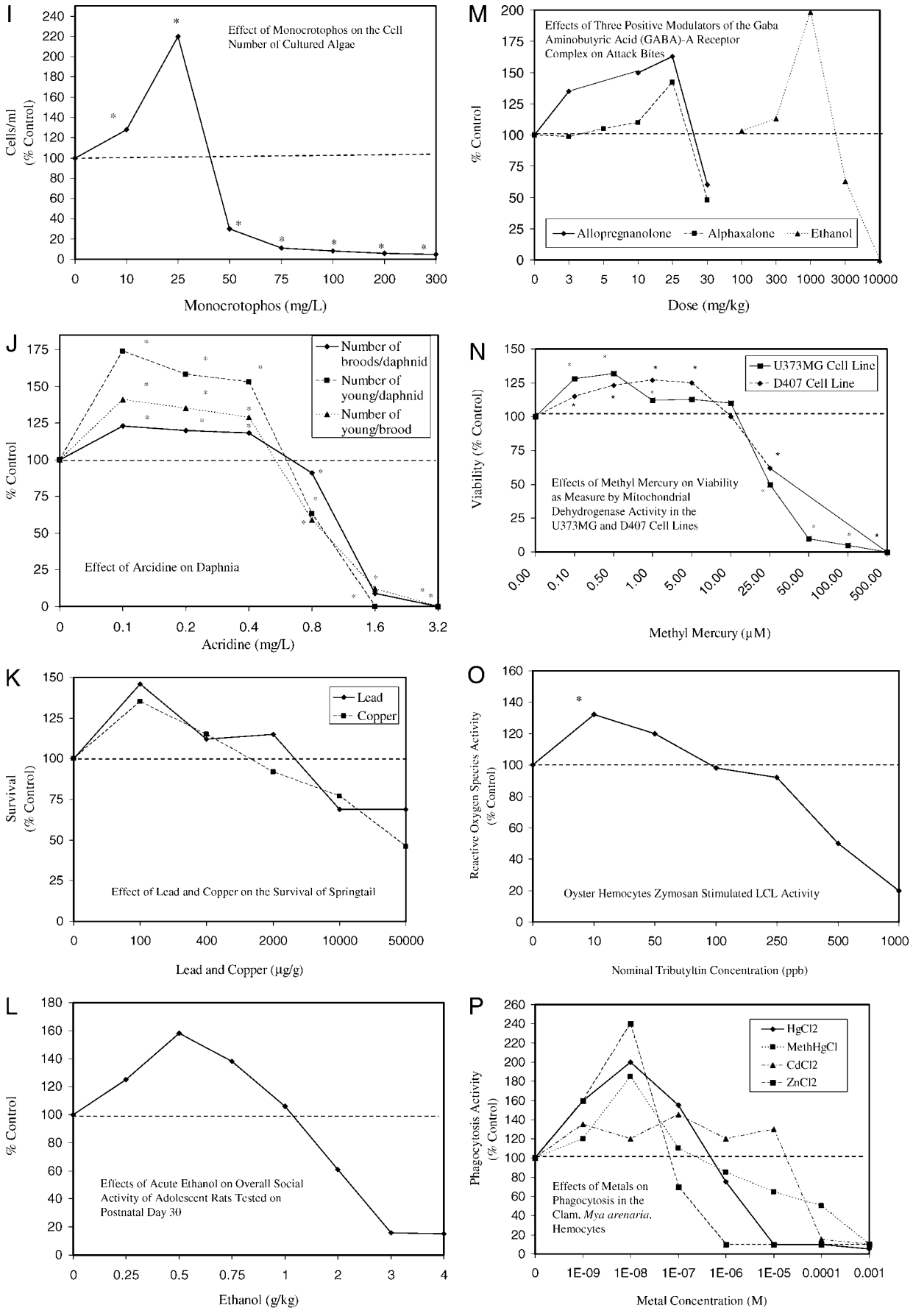


Fig. 3. Continued.

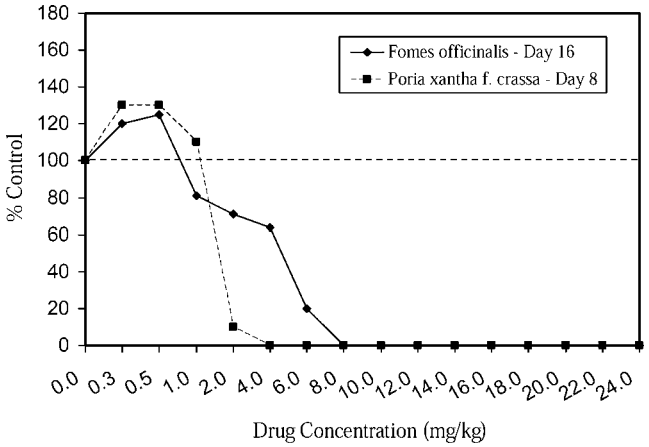


Fig. 4. Percentage of normal growth on malt agar containing various concentrations of western red-cedar heartwood extract on selected fungal species [15].

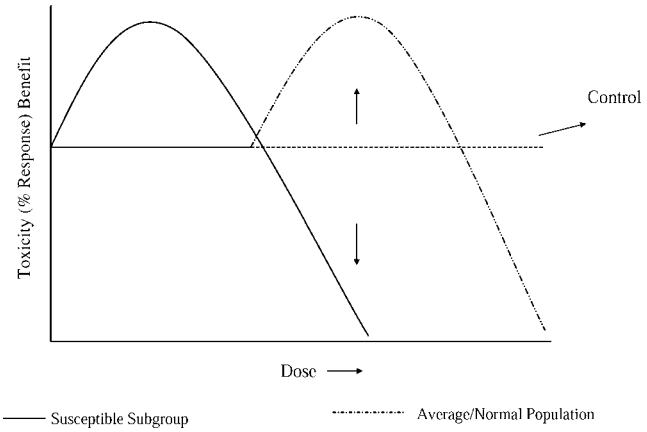


Fig. 6. Hormesis and differential susceptibility.

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

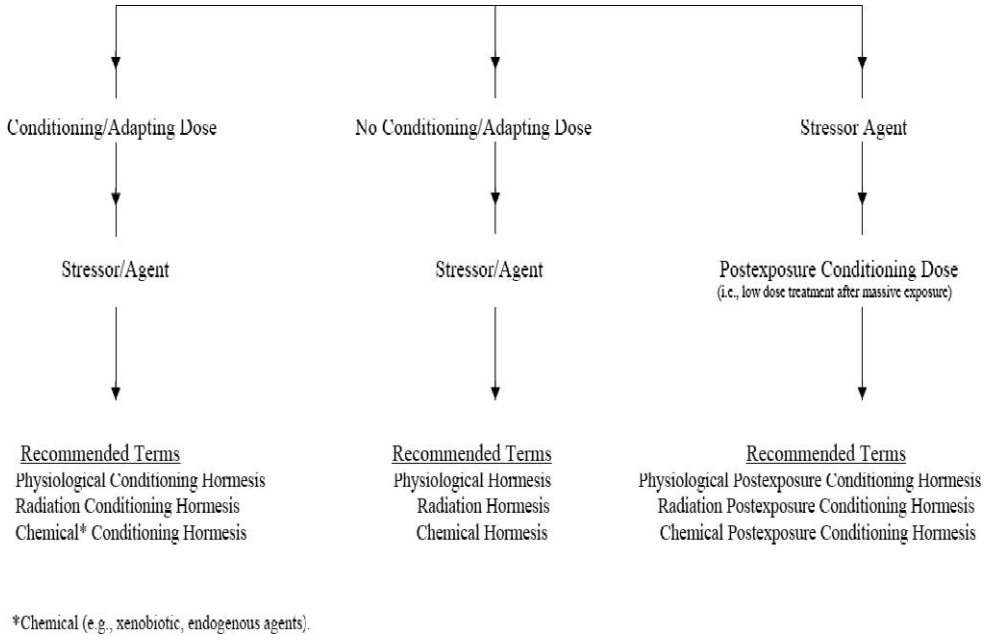


Fig. 5. Summary of biological stress terminology. Preconditioning, adaptive response, and autoprotection represent examples of what is described here as conditioning hormesis. Advantages include the following: Standardized terminology provides information regarding the presence or absence of a conditioning 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].

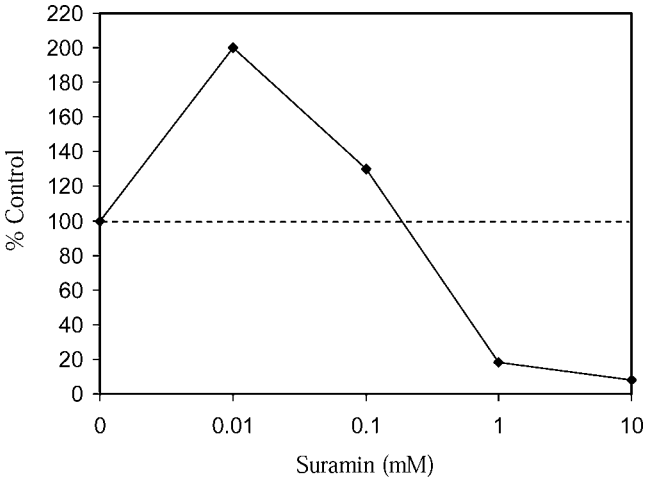


Fig. 7. Effect on prostate cancer cell (MLL) growth [2,184].

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.

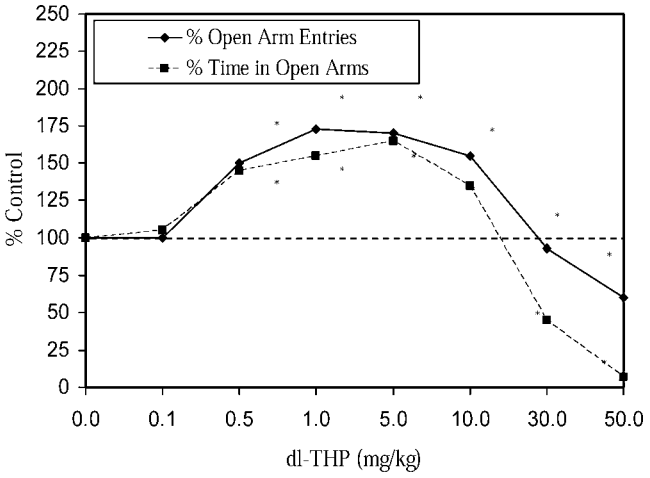


Fig. 8. Anxiolytic effect of dextrorotatory levorotatory–tetrahydropalmatine (DL-THP), a naturally occurring alkaloid, on Institute of Cancer Research (ICR) mice of both sexes in the elevated plus-maze test. An asterisk indicates a significant difference from the control ($p < 0.05$) [21,185].

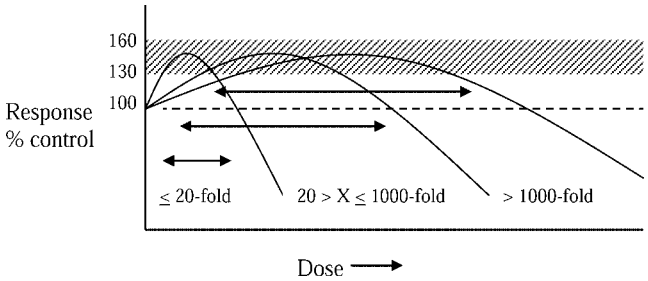


Fig. 9. Dose–response curves indicating the relative distribution of stimulatory dose ranges. The shaded area represents the maximum stimulatory response range, which typically is 130 to 160% of the control value.

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].

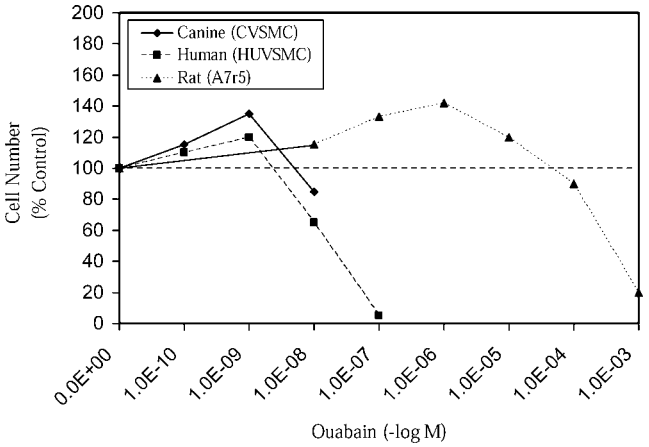


Fig. 10. Effects of ouabain on proliferation [44].

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

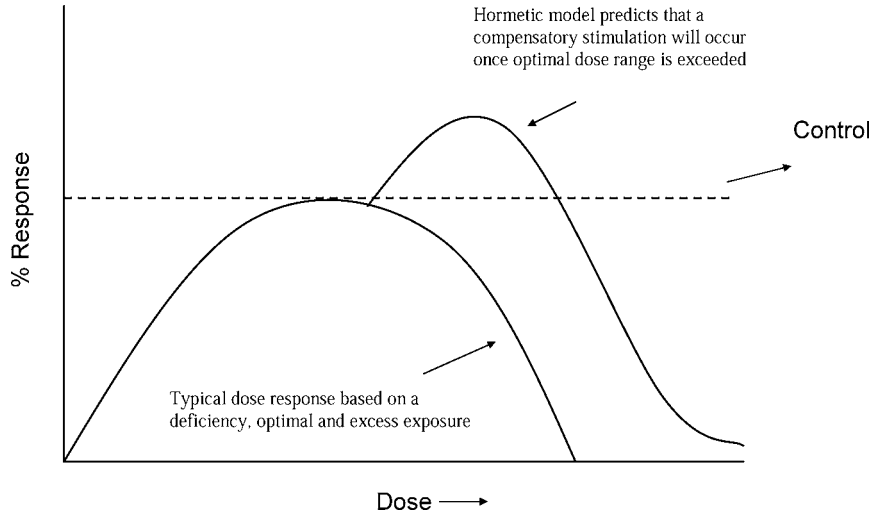


Fig. 11. Hypothetical hormetic dose–response relationship for a nutrient.

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

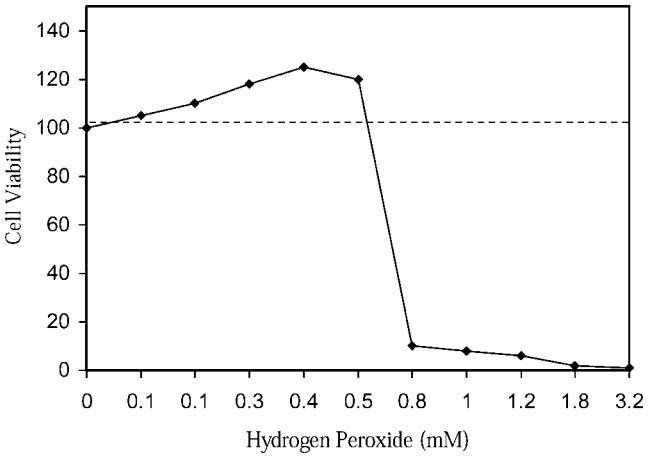


Fig. 12. Effect of hydrogen peroxide on cell viability of *Saccharomyces cerevisciae* strain R253 [96].

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.

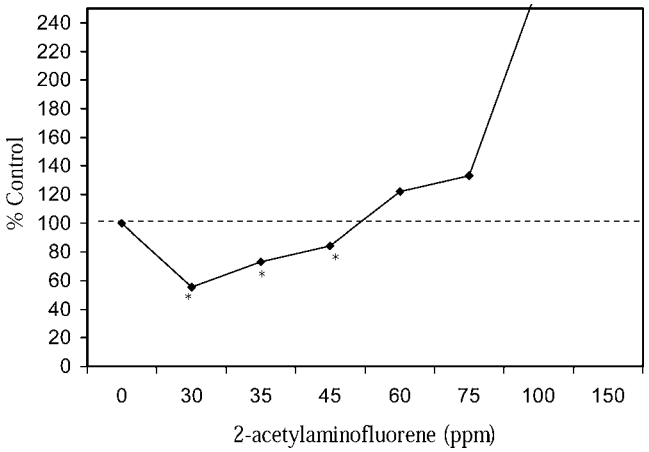


Fig. 13. Incidence of bladder tumor adjusted for time in ED01 megamouse study [1,121].

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.

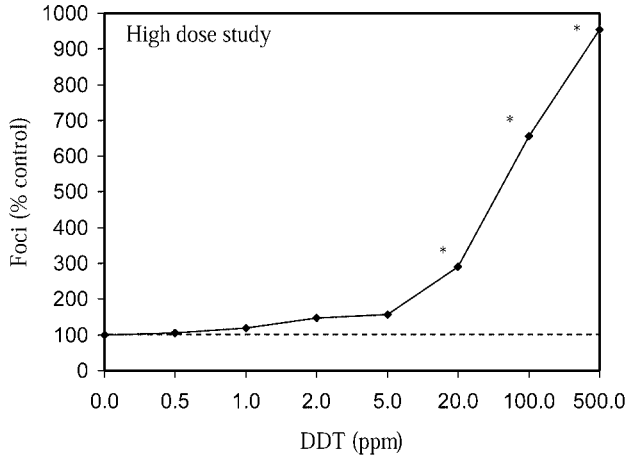
The 2-AAF study, now referred as the megamouse study or the ED01 study, was resource intensive and may never be undertaken again with rodents. In this one-of-a-kind study, however, using a model carcinogen and involving enormous previous planning to ensure adequate testing, the hormetic dose response 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



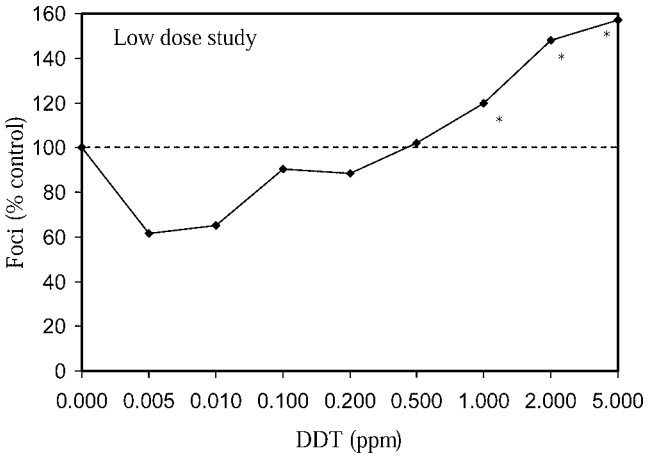


Fig. 14. Effect of DDT on number of glutathione-*S*-transferase P-positive foci in F344 rat livers in bioassays assessing different but slightly overlapping doses of carcinogen. Note that as the dose decreases, the J-shaped dose–response curve becomes evident. Also, note the difference in scale between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

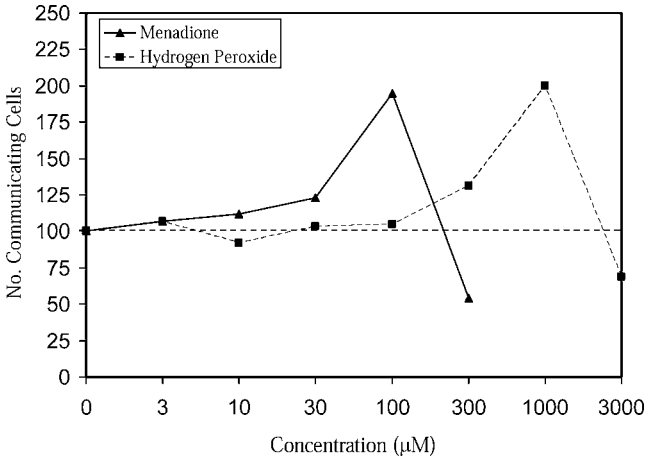


Fig. 15. Gap junction intercellular communication (GJIC) levels in BPNi cells exposed for 1 h [123].

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*

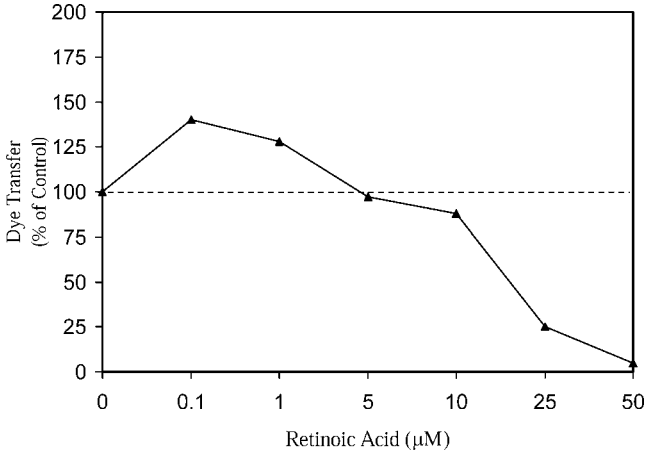


Fig. 16. Effects of retinoic acid on dye transfer in IAR 203 cells [125].

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.

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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

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Critical Review

TOXICOLOGY REWRITES ITS HISTORY AND RETHINKS ITS FUTURE: GIVING EQUAL FOCUS TO BOTH HARMFUL AND BENEFICIAL EFFECTS

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Abstract—This paper assesses how medicine adopted the threshold dose–response to evaluate health effects of drugs and chemicals throughout the 20th century to the present. Homeopathy first adopted the biphasic dose–response, making it an explanatory principle. Medicine used its influence to discredit the biphasic dose–response model to harm homeopathy and to promote its alternative, the threshold dose–response. However, it failed to validate the capacity of its model to make accurate predictions in the low-dose zone. Recent attempts to validate the threshold dose–response indicate that it poorly predicts responses below the threshold. The long marginalized biphasic/hormetic dose–response model made accurate predictions in these validation studies. The failure to accept the possibility of the hormetic-biphasic dose–response during toxicology's dose–response concept formative period, while adopting the threshold model, and later the linear no-threshold model for carcinogens, led toxicology to adopt a hazard assessment process that involved testing only a few very high doses. This created the framework that toxicology was a discipline that only studied harmful responses, ignoring the possibility of benefit at low doses by the induction of adaptive mechanisms. Toxicology needs to assess the entire dose–response continuum, incorporating both harmful and beneficial effects into the risk assessment process. Environ. Toxicol. Chem. 2011;30:2658–2673. © 2011 SETAC

Keywords—Hormesis Hormetic Biphasic Dose–response Risk assessment

INTRODUCTION

To claim that the scientific discipline of medicine got the dose–response wrong and with this error damaged our health, environment, and economy sounds wrong, irresponsible, and unfair to such a dignified and life-serving profession. This accusation seems right off the pages of an attention-grabbing national tabloid one may often see while waiting in line to pay for groceries, rather than an academic appraisal. The problem is that the accusation has a compelling and detailed historical record, a record that has taken more than two decades to unravel and reconstruct. This story is a historical detective adventure, initially set in northern Germany in the modestly sized academic city of Greifswald during the latter decades of the 19th century, later transforming into an international affair, reaching the highest levels of government in the United States and in multiple European countries, with documentation of its continuing international presence in the control of legislation, major governmental programs, university curricula, as well as the lives of citizens and their health judgments [1–3].

Although many articles and book chapters have been written on the history of toxicology and pharmacology, the perspective offered here is unique. The present paper contends that the most fundamental principle of toxicology and pharmacology, the dose–response relationship, arose out of a dispute between two intense professional rival organizations—traditional medicine and homeopathy. It will be shown that homeopathy was the first of the two organizations to claim that what they believed was the most fundamental nature of the dose–response: asserting it to be the biphasic dose–response, later to be called hormesis. Having lost the benefit of first discovery or proclamation, traditional medicine led a powerful, prolonged, and unrelenting attack on this dose–response concept and its formulator, Hugo Schulz (1853–1932), all in an effort to discredit the model (which Schulz called a law) and their medical opponent, homeopathy. In time, leaders within traditional medicine then proposed their dose–response alternative (the threshold dose–response model) and used their influence to get it established at all levels of scientific society, including government regulatory agencies, academic institutions, the chemical and pharmaceutical industries, and professional societies. As will be seen, they were amazingly successful, but at a very high cost, for in their quest for victory over homeopathy, they failed to validate their dose–response model in the critical low-dose zone, where most human exposures to drugs and chemicals occur.

The claim

Modern medicine is the parent of pharmacology, which begat toxicology, which then produced risk assessment and risk communication, all products of the 20th century. The fields of pharmacology and its then nascent offspring, toxicology, adopted the threshold dose–response model in the 1930s, convincing governmental regulatory agencies to incorporate it into all subsequent regulations for chemicals and drugs. The leaders of these disciplines, both in and out of government, never attempted to validate this model for accurate predictions, where people live, that is, in the low-dose zone, below the toxicological threshold, nor were they asked to do so by generations of legislative leaders and their scientific advisors. At the core of this issue is the denial by these medically dominated fields of the very existence of an alternative dose–response model, called hormesis, from a rival medical concept/organization called homeopathy.

The hormesis concept has long been marginalized in multiple reinforcing ways by the scientific and medical communities.

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This may be inferred by its long exclusion from the leading textbooks of medicine, pharmacology, and toxicology and, as a consequence, its lack of presence in academic curricula and the classroom. Professional societies have likewise denied hormesis a presence at their annual and regional meetings, thereby preventing professional attendees from getting the opportunity to learn about this concept and to observe research presentations on the topic. Of further significance is that the hormesis concept has long been excluded from governmental grant funding processes, a tactic that would ensure an untenured professor academic failure if he or she pursued a possible hormetic area of research interest. The hormesis concept also has been excluded from affecting the development of environmental and medically related legislation, which develop broad-reaching policies, direct the flow of money and resources, and influence a vast array of human behaviors. In effect, modern medicine and its pharmacological and toxicological offspring incorporated an unproven and nonvalidated dose–response model, called the threshold dose–response, into its profession, made it their gold standard (default) model, while purposefully and severely marginalizing its opponent's model (hormesis), letting it function as a historical artifact of a discredited medical practice and thus giving the hormesis concept the equivalent of a professional death sentence.

These medically related fields passed on the threshold dose–response model and its hazard assessment testing and risk assessment schemes to ever newer generations of pharmacologists, toxicologists, risk assessors, and risk communicators. These scientists, physicians, and social scientists knew little of their history and even less of the dose–response machinations by their medical grandparents. They too were unaware that they had been professionally misled and manipulated with the firm expectation that they would do the same to their students as was done to them. This concept orchestration and data censoring by traditional medicine and its disciplinary descendants would occur, and continue to occur, in ostensibly free societies, where people, including the scientific community, were led to believe that they were in control of what to accept or not. They were, however, unaware that political, economic, and pervasive institutional (medicine, academic, governmental regulatory agencies) forces converged to suppress the hormesis concept while promoting an alternative or rival model, the threshold dose–response. Now let us examine what this claim is based on.

The medical rivalry—its unintended consequences

It is now the second decade of the 21st century. While the mid-19th century seems quite distant from today, history has a long reach. Consider race relations in the United States. The United States is still feeling the effects of a Constitution in which Blacks were considered three-fifths of a person for census information and Congressional representation. The U.S. Civil War from 1861 to 1865 was a national trauma beyond comprehension. Even a century later, the country was still trying to figure out how to ensure that African Americans would have equal access to education, jobs, health care, housing, restaurants, and other social and professional venues. History is important; it has deep and entangling roots that often impact the present and future in ways that may not be obvious, but nonetheless are real, powerful, and controlling.

Pharmacology and toxicology had their roots in what we now call traditional medicine. Traditional medicine emerged from what is generally referred to as the era of heroic medicine, when physicians of the 18th and 19th centuries often treated patients rather harshly with blood drawings, bloodsucking leaches, and highly toxic agents such as arsenic and mercury, while often conning patients into purchasing and ingesting elixirs that could have caused harm. As many may know, the death of George Washington in 1799 was probably accelerated by repeated and extensive blood drawings to cure him of what is now thought to have been a bacterial infection (www.eyewitnesstohistory.com/washington.htm). Furthermore, the only daughter of John Adams, the second president of the fledging United States, underwent surgery for breast cancer without an anesthetic (www.shsu.edu/~pin_www/T@S/2002/NabbyAdamsEssay.html). It was pretty clear that in the days of heroic medicine, one was fortunate to endure and survive a host of what could only be called barbaric treatments. This statement does not even consider the effects on family members who watched and had to deal with suffering directly related to such heroic medicine practices. Thoughtful physicians of the day often recognized that they were far from being healers but often were the equivalent of an unwilling but necessary torturer. One of these conscience-riddled physicians of the late 18th century who just could not "take it anymore" was Samuel Hahnemann (1755–1843), a very bright German, who sought and created an alternative to the practice of torturous heroic medicine [4–6].

Hahnemann challenged the establishment by creating the medical practice of homeopathy, an action somewhat akin to the earlier machinations of Martin Luther when he posted his Ninety-Five Theses in 1517, which eventually led to the creation of the first Protestant church. Let us put it this way: Martin Luther's challenge did not go over well with the Pope and his bishops; the actions of Hahnemann created a similar set of enemies, only his were in medicine, not theology. In both cases, the stakes were high. On the plane of idealism, Hahnemann's fight was about life and death in this world, whereas in the case of the church, it was about death and life in the next. In the world of pragmatism, this intense competition was also about power, politics, influence, money and, of course, control. In addition, Hahnemann was not easy to like. Brilliant though he probably was, he offered an equal dose of bitter and arrogant invective that simply fueled the conflict, creating a long line of personal enemies just awaiting their time for payback. Few attempts were made at compromise. Whether homeopathy cured patients really was not the issue, at least at the time of Hahnemann, and in fact, has nothing to do with the premise of the present paper. The scheme worked out by Hahnemann of using extremely dilute doses of plant-derived extracts as homeopathic drugs was at least not very likely to injure his patients. In this dimension, homeopathy was probably superior to heroic medicine. It did not torture its patients or speed them along to an early grave, and it may even have provided a healing boost if only via a placebo effect. Indeed, homeopathy was winning the hearts and minds of many adults in Europe and the United States, and, of course, gaining an ever-greater market share [5,7].

The battle between homeopathy and traditional medicine has been longstanding [5,7], the stuff of bitter internecine hostilities, much like warring political parties, opposing churches, or even the heated family feud, in which ghastly homicides can occur. The battles could be intense. It was about which profession was going to win in this most important aspect of life. As with the U.S. civil war, the traditional medicine–homeopathy conflict also has had a long reach that is as intriguing as it is important. It will now be shown how its outcome has profoundly affected the development of toxicology and pharmacology, the testing and safety of drugs and chemicals, the risk assessment process, the risk communication message, as well as

the entire range of environmental health exposure standards for all environmental (e.g., air, water, food, soil, consumer products) media.

Schulz's mistake: Biphasic dose–response to homeopathy

In February 1884, the physician and pharmacologist Hugo Schulz first presented evidence of the biphasic dose–response (to be called *hormesis* in 1943 by Southam and Ehrlich [8]), based on experiments assessing the effects of disinfectants on yeast metabolism, at a meeting of the Greifswald Medical Association (http://www.Medizin.uni-greifswald.de/medverein/Geschichte.htm) and subsequently published his findings [9,10]. The low-dose stimulatory response was a surprising observation of which Schulz was initially skeptical. However, repeated successful replication experiments led him to be confident that the observed biphasic dose–response was highly reproducible and extended to a wide range of chemical disinfectants ([11]; translation of the Schulz, 1923 autobiography, [12]).

Schulz used these findings to explain a striking series of clinical observations by Bloedau in 1884 ([13]; cited in Schulz [14]), in which a homeopathic preparation (veratrine) was used to successfully treat gastroenteritis. Schulz was so intrigued with these clinical findings that he tested whether such a preparation would directly kill the recently isolated causal bacterium of this disease. However, his experiments indicated that the veratrine was unable to do so, regardless of the dose applied [14]. Although these experimental findings could have led Schulz to conclude that the homeopathic preparation was not an effective treatment of gastroenteritis, they did not. In fact, Schulz hypothesized that the homeopathic treatment was effective but that its mechanism was not directly bactericidal but via the induction of an adaptive response in the patient to resist the infection. After conversations with his colleague Rudolph Arndt, Schulz linked this adaptation hypothesis with his biphasic dose–response observations in the yeast. He then proposed that the low-dose stimulation represented an adaptive process and that this was how low but measurable doses (i.e., not an ultra low, extremely high dilution dose below Avogadro's number) of homeopathic preparations worked. At this point, Schulz came to believe that he had discovered the explanatory principle of homeopathy, later naming it the Arndt-Schulz Law.

Schulz's gift to homeopathy directly led it to become the first of the medical professions to stake a claim on the nature of the dose–response, especially in the low-dose zone. When seen through the lens of history, homeopathy scooped traditional medicine on the key issue of the dose–response and its potential for drug development and patient treatment.

The scooping of traditional medicine on the nature of the dose–response was no small accomplishment for the underdog homeopathy, beating it to the punch on the critical pillar of their profession. Whether anyone at the time truly appreciated the significance of this achievement is not clear. However, the fact that Schulz quickly became an object of vicious criticism and professional ridicule by his medical colleagues suggests that the leaders of the traditional medicine movement understood only too well what was at stake [12]. In his autobiography, Schulz [11] recounted in a striking way how he incurred professional ostracism by his traditional medical colleagues, indicating how he was viewed with suspicion because of his research on homeopathy. He also became the object of a derisive writing campaign that referred to him as the Greifswald Homeopath. These changes in professional relationships occurred soon after his 1885 publication [14] that proposed a low-dose adaptive response mechanism for the homeopathic preparation veratrine, in effect excluding him from their group. So polarized was the relationship between traditional medicine and homeopathy that Schulz remained an outsider and the object of continuing criticism and judgmental actions for his entire nearly 50-year academic career. In fact, on the occasion of Schulz's retirement, Martius-Rostock [15] reflected on his long and conflicted career, lamenting "What law did Hugo Schulz break that makes him deserving of the boycott exercised by his scientific peers?" He additionally criticized those who distorted Schulz's image "through incomprehensible, idle talk." Schulz's biphasic dose–response and its meaning would soon be challenged and denigrated on multiple levels, and he and his supportive colleagues, such as the eminent August Beir (the father of spinal anesthesia) (1861–1949) [16], along with it.

The dose–response "take back" by the medical profession was led by Alfred J. Clark (1885–1941), a professor of pharmacology at the University of Edinburgh, who occupied the most coveted academic position in pharmacology in Europe. Clark had worked his way up through the ranks, with professorships in South Africa (1918–1920), London (1920–1926), and Edinburgh (1926–1941). Along the way, he established himself as an expert in quantitative pharmacology. He was able to combine excellent mathematical skills with his training in medicine and pharmacology and was clearly smarter and more focused than even a normally gifted contemporary physician and pharmacological researcher. His unique combination of skills and drive gave him a clear edge on his peers and enemies. Clark also was a meticulous researcher with a flair for writing. He applied his prodigious skills to the shaping of the field. By virtue of his highly successful textbooks [17–20], he taught pharmacologists and toxicologists for approximately a half century, well after his untimely death in 1941 [21,22]. In the foreword to the book *Towards Understanding Receptors*, Robinson [23] referred to the 1937 text by Clark as the "now classic monograph on *General Pharmacology*, a book that had great influence on a number of individuals." Clark not only used these textbooks to teach pharmacology and toxicology, but also as a vehicle to emasculate homeopathy, Hahnemann, Schulz, and his Arndt-Schulz Law, that is, the hormesis concept (Appendix 1).

The new medicine man: The threshold model

The threshold dose–response model became medicine's alternative to the biphasic dose–response of homeopathy and Schulz. This model would become the driver for therapeutic medicine for the rest of the century and beyond. How Clark achieved his historical milestone of dose–response control is now described. Clark was a first-rate scholar. His textbooks were thorough, detailed, and second to none, at least for his era and the next generation. This was no small accomplishment, and he had the admiration of many. He never left a scientific stone unturned, so to speak. This high level of detailed professionalism brought him to the leadership of his field, along with a likeable, highly principled personality. It also made him the ideal scientific candidate to attack and discredit homeopathy and Schulz. Thus, the fact that Clark failed to present, discuss, and at least try to refute the substantial body of research that supported Schulz's dose–response model, especially when it had been broadly reported, in excellent journals and by scientists of high visibility and accomplishment [24], is highly surprising. Not that Clark did not have a sense for the scientific literature or how to obtain and analyze it—his textbooks and other writings amply illustrate that he was among the best when

it came to digging out information from even the most obscure sources and integrating apparently disparate information into plausible biomedical theory.

Evidence to support this conclusion may be seen in Clark's selective use of references to refute Schulz. For example, his influential text entitled *The Mode of Action of Drugs on Cells* [18], citing Dannenberg [25], argued that Schulz's low-dose stimulatory responses in yeast were attributable to background variation/experimental error, not real treatment effects. Based on these findings and interpretations, Clark then dismissed both the plausibility and the biological significance of the Arndt-Schulz Law. What Clark [18] did not mention was much more significant. First, he failed to show that the doses used in the Dannenberg [25] study were far below those known to cause stimulatory responses in the earlier yeast studies; the highest doses were some 10-fold to 20-fold below the lowest doses associated with such stimulation. Also not mentioned was that the Dannenberg [25] study assessed responses at only a single time point, thereby eliminating the possibility of observing an overcompensation stimulatory response. Clark [18] also neglected to cite studies that supported the Schulz findings, including a recent detailed investigation by Branham [26], which was published in the highly visible *Journal of Bacteriology*. This study was specifically designed to replicate and extend the research of Schulz on the effects of chemical disinfectants on yeast metabolism. Branham [26] tested a similar broad spectrum of agents as used by Schulz (not simply three as reported by Dannenberg [25]), incorporated a detailed dose time component, and assessed responses over a very broad dose range that included doses both above and below the toxicity threshold. Her findings strikingly supported the observations of Schulz, clearly documenting the low-dose stimulation and high-dose inhibition, while revealing that the stimulatory responses resulted from an overcompensation to an initial disruption in homeostasis (that is, toxicity). A dose-dependent toxicity occurred at the first time point, followed by the overcompensation stimulatory effect. The stimulatory effects were also 40 to 80% greater than the control group response. The study of Dannenberg [25], which Clark [18] so highly relied on, was therefore clearly not designed to fairly test the Schulz hypothesis, whereas the Branham [26] study was.

My colleague Linda Baldwin and I further documented this type of scientific misrepresentation in five publications, detailing the historical foundations of hormesis in the biological and biomedical literature, occupying an entire issue of the journal *Human and Experimental Toxicology* [27–31]. Although considerable support was shown for the hormesis concept in the early decades of the 20th century in chemical toxicology, pharmacology, and radiation biology, these findings were also neglected by Clark. The hormetic-biphasic findings were broadly generalizable, often substantial research contributions, and readily obtainable, even without electronic databases. The failure of Clark to recognize and address these studies supporting the hormesis/biphasic dose–response perspective was very damaging to Schulz, adversely affecting the acceptance and utility of his body of work, including his dose–response concept, the Arndt-Schulz Law, as well as the development of pharmacology, toxicology, environmental health, and risk assessment.

As a result, many academicians and researchers in the fields of pharmacology and toxicology succumbed to an appeal to authority. For if Clark could not find support for the biphasic dose–response—he of such high regard, the leading professor of pharmacology among a bevy of other outstanding professors, an influential governmental advisor, the masterful researcher and textbook writer, a person with a reputation for being objective, fair-minded, comprehensive, and totally professional—then the support for Schulz's model could not have been convincing. In effect, the fields of pharmacology and toxicology allowed Clark to do their thinking on this critical issue.

Clark was a man of great accomplishment and continues to be held in high regard. The University of Edinburgh has a distinguished chair in his name, and graduate fellowships in his honor are awarded by the British Pharmacological Society. These recognitions and honors are not given out lightly or often; they are earned. Nonetheless, despite these honors, Clark failed the scientific community on the most critical and far-reaching concept, the nature of the dose–response.

The threshold dose–response: Historical foundations

The threshold model was not unreasonable; it seemed consistent with much published data and was a concept that resonated with personal experience and common sense. In fact, this may have been why Schulz initially doubted his biphasic dose–response observations when first they appeared in his experiments with yeast. The threshold dose–response concept is believed to have been originally put forward by the legendary French biologist Claude Bernard (1813–1878) [32] within the context of the excretion of glucose. Others extended this concept to the excretion of additional pharmacological/physiological agents such as chloride, urea, and other metabolic products [33–38]. The threshold perspective was then placed within a more general context by Cushny (1866–1926) [39], who developed a simple formula-based model to describe the threshold response. Clark [40] also had some experience in the study of dose–responses, observing a biological threshold for acetylcholine that required approximately 20,000 molecules acting via receptors to produce an initial effect on a heart cell (e.g., isotonic contraction). Clark had been a professor working in Cushny's department and twice replaced him as Department Chair (at the University of London, 1920, and then at Edinburgh, after Cushny's death in 1926) [41,42]. Such research on the threshold concept was further extended in the laboratory of the Nobel Prize winner and British pharmacologist Charles Scott Sherrington, by Russell Aitken and his advisor J.G. Priestley [33]. Although the research of Aitken [33], Cushny [39], and others was in the pharmacological domain, support was also offered in the toxicological [43–45], radiation/occupational health [46–48], and immunological [49] areas for the generalizing of the threshold dose–response concept. Thresholds were also widely observed in numerous other scientific domains, ranging from the behavioral to the physical sciences, supporting a broad and integrative general scientific concept [50]. No need was felt to consider the Schulz alternative dose–response concept.

The anatomy of a scientific takeover

Just how did the threshold dose–response concept get established?

Step 1: Challenge the alternative model

Clark's various publications on the quantitative features of pharmacology and toxicology had both a devastating and lasting impact on Schulz and the hormetic dose–response. So too did his attempts to reach the broader biomedical community, in highly respected journals, such as the *British Medical Journal* [51]. In this journal and in other writings, he lumped homeopathy with numerous garden-variety versions of medical witch-

craft. In this process, he was broadly successful in creating a repeated focus on homeopathy, that is, his real target, emphasizing its high dilution aspects, calling it quackery and then associating Schulz and his work with it. Clark's mistake was that Schulz did not adhere to the high dilution school of homeopathy [12,52], the only segment of homeopathy that Clark addressed. The written contemporary record was clear that Schulz did not support high-dilution homeopathy in theory or practice. High-dilution homeopathy refers to homeopathic practices in which the dose of a therapeutic agent is diluted to such an extent that there are likely no molecules of agent in the medical treatment. The fact that Schulz offered an explanatory principle for homeopathy based on his biphasic adaptive dose–response while rejecting homeopathy's high dilution features made him both a leader and controversial figure in the homeopathy domain. These points were clearly documented while he was alive and broadly expressed in his obituaries in the homeopathic and traditional medicine literature. For example, Paul Wels, an eminent radiation biologist (Schulz died on July 13, 1932) presented a remembrance lecture on Schulz at a meeting of the Greifswald Medical Society on November 5, 1932. In this presentation, entitled "The Life Time Work of Hugo Schulz", which was published in 1933 [53] in a leading pharmacology journal, Wels stated that "Schulz gave the dosage question its entitled place while homeopathy made it laughable." By unfairly linking Schulz to high-dilution homeopathy, Clark sought to undercut his credibility so he would not be taken seriously by the scientific community.

So strong was the leadership of Clark in the domain of preserving the integrity of traditional medicine via his attacks on its opponents like homeopathy and Schulz that this very point was emphasized in eulogies after his own death and were summarized by his physician–psychiatrist son, David Clark (1920–2009), in an insightful biography. That is, he was remembered positively by even Nobel Prize recipients (e.g., Sir Henry Dale, 1936 recipient of the Nobel Prize for biology and medicine) for his key role in the rapid downturn of homeopathy and other forms of quackery [41].

Step 2: Propose your model, get it accepted

The period of concept consolidation involved extensive support for the threshold model in the numerous publications by Clark and his contemporaries. This served as a basis to establish the necessary peer-review–based credibility for the threshold model. Given his mathematical background, Clark recognized the value of integrating his dose–response concept within a biostatistical framework using the newly developed probit dose–response model of two of his colleagues in the early to mid-1930s [54,55]. This mathematical model was derived independently by John Henry Gaddum (1900–1965), another extremely gifted quantitative pharmacologist, and Chester Bliss (1899–1979), an itinerant, yet highly productive, biostatistician who was befriended by Clark at a critical time in his career. As we shall see, Bliss repaid Clark many times over for his professional and personal support. The probit dose–response model then received a major endorsement and a key provision, both provided by the world-renowned and later to be knighted Ronald A. Fisher (1890–1962). Fisher added a procedure to the model called the maximum likelihood estimate as an appendix to the key 1935 paper of Bliss [54]. The monotonic features of the probit model were employed to constrain predicted responses to asymptotically approach the control value at low doses while never being permitted to transition below the control as would occur in the hormetic-biphasic dose–response model of Schulz. The significance of such a biostatistical manipulation was that it denied the existence of the hormetic dose–response. Dips in the response below the control surely did occur. However, this was assumed to be only attributable to response variability. It was not real in the sense that the findings were reproducible; thus, the best estimate could never be below the control group. The hormesis idea was not only marginalized; it was not considered to have biological credibility. This was the unmistakable take-home message from the intellectual biomedical leadership of that critical concept-defining era.

Step 3: Make your model the standard procedure

This intellectual fusion of the best, brightest, and the most influential biomedical leaders of the day successfully consolidated the threshold dose–response concept into the mainstream of pharmacology and toxicology. Because Clark was also part of the broader elite that created the British Pharmacological Society in 1929, this provided him with ready access to and acceptance by essentially all professors of pharmacology within the entire UK system and their extended pharmacological families in other countries, including the United States, and their journal publication vehicles. This was especially true for the United States, because most serious graduate students viewed a European graduate education experience during this period as a key to obtaining an excellent education, establishing a broad network of professional contacts, and a subsequent position at a leading U.S. academic institution [56]. This was commonly the case for many disciplines, including chemistry, microbiology, botany, and, of course, pharmacology.

The highly positive view of British pharmacological academic elites made acceptance of the Clark perspective on the dose–response even more efficient internationally. Clark's goal of establishing his model yielded rapid success. He had the concept, the textbooks, and the network with its coordinated activities and influence, with no competition or credible opposition.

Success with his pharmacological colleagues, as important as that was, however, was not enough. Numerous other biological subdisciplines had to be educated on the nature of the dose–response, so that medicine could assert dominance of its model in all of biology and the biomedical sciences. This next phase of concept integration was under the per-view of Clark's colleague, Chester Bliss, who wrote a series of publications for the most prominent journals of biological disciplines concerned with dose–response relationships, such as microbiology, entomology, food science, radiation biology, and others [57–62]. These publications, which describe the nature of the dose–response at low dose as well as how to quantitatively assess, interpret, and apply such findings, further ensured the broad acceptance of Clark's perspective. In effect, Bliss sent different versions of the same conceptual paper to many biological subdisciplines, but tailored to the readership of each area. In his writings, Bliss established the term *threshold dose,* defined it, provided various means to estimate it, and integrated it into the mass-action formula used by Clark [18,57], leaving no room for confusion, debate, or compromise. Bliss was absolutely tireless in getting the dose–response message out, ensuring that it influenced the educational process of most students being professionally trained in the biological sciences, later to become the leaders of academia and governmental agencies. Bliss was inspired to continue this educational process during the years before and after the death of Clark. Although Bliss has never been seen as a major player in the course of mid-20th century

science, he nonetheless may well have had the greatest impact for enhancing the implementation of Clark's dose–response perspectives (at the expense of hormesis) into graduate training during the last half of the 20th century. Without Chester Bliss, the potency slope for the acceptance of Clark's dose–response concept would have been much flatter.

Lasting legacy

The biostatistical constraining of the dose–response to approach the control value only asymptotically became a major factor in the regulation of exposures to carcinogens. In the early 1940s, the U.S. National Cancer Institute showed how this would be implemented for a chemical carcinogen. In a study in which the data demonstrated support for an hormetic dose–response relationship for a carcinogenic polycyclic aromatic hydrocarbon, the investigators determined that this agent could not possibly display a risk of disease below that of the control group [63]. They then followed the constraining monotonicity of the probit model, eliminating the possibility of a hormetic dose–response. This constraining concept was later to become policy in the Food and Drug Administration (FDA) and the U.S. Environmental Protection Agency (U.S. EPA) for carcinogen risk assessment and remains so today.

In the early 1950s, one of the famous and most endearing forefathers of U.S. regulatory toxicology, the late Arnold Lehmann (1900–1979), created the framework for the use of safety factors, also called uncertainty factors. This safety factor procedure was easily understood, implemented, and copied by all regulatory agencies in the United States and worldwide.

What Lehmann accomplished was significant. He gave the regulatory world the safety factor. It was based on the assumption of a threshold dose–response model, compliments of A.J. Clark, on whose textbooks Lehmann had been reared academically. In retrospect, Lehman and his colleagues, following the example of Clark, failed to validate the threshold dose–response model at this most critical juncture of regulatory science history, thereby contributing to toxicology's failure of due diligence.

Linearity wins a seat at the risk assessment table

As much as the threshold model dominated the second half of the 20th century, it had its detractors in the biomedical community. These detractors were not those considered on the fringe, the so-called quacks from the long since defeated and marginalized homeopathy community. They were a fledgling group of brilliant geneticists focused on mutations, led by an insightful, but sometimes hard to fathom, academic and later Nobel Prize winner by the name of Hermann Muller (1890–1967). After training with the future Nobel Prize winner Thomas Hunt Morgan (1866–1945) at Columbia University in the 1910s, life took on added intensity for Muller in the mid-late 1920s in his quest to establish that ionizing radiation could cause mutations. Through a series of intriguing experiments at the University of Texas at Austin, Muller uncovered one of the geneticists' holy grails: X-rays could cause mutations in the gonads of male fruit flies [64]. The surprising aspect of Muller's publication was that it contained no data. In fact, the publication was an oddity, amounting to a detailed discussion of unreported data. The article in *Science* aroused debate and confusion, leading former advisor Thomas Hunt Morgan to proclaim, "Now he's done it. He's hung himself" [65]. Lacking methods and data made Muller's *Science* paper suspect. The suspense ended when Muller presented the actual data behind the paper at the Fifth International Genetics Conference in Berlin later that year and subsequently published in the conference proceedings [66]. It took 19 years for Muller's dream of a Nobel Prize to come true. However, by 1946 the world was frightened by the bomb, its unique capacity for massive devastation, and its potential to cause all sorts of genetic diseases in future generations. Thus, on December 12, 1946 Muller ([67]; http://www.nobelprize.org/nobel_prizes/medicine/laureates/1946/muller-lecture.html) was awarded the Nobel Prize and in his acceptance speech gave further credibility to the field of genetics, while rallying his geneticist colleagues to the belief that only they could save the world from the harmful effects of ionizing radiation [68].

The National Academy of Sciences

The leaders of the genetics community feared that radioactive fallout from atmospheric atomic bomb testing had the potential to threaten the health of future generations of humans, in the United States and elsewhere, by causing mutations in reproductive cells. Working through various national and international committees, they tried to convince the more medically trained committee members that there was no safe dose of ionizing radiation. They argued that radiation would act in a linear fashion and that the risks were inescapable no matter how low the exposure. The geneticists were united in arguing that radiation-induced birth defects would increase significantly because of the atmospheric testing of atomic bombs. While they were on the losing side of a number of key national and international advisory committee recommendations, they won the big one [68]. That is, things changed in 1956 during the deliberations of the BEAR I (Biological Effects of Atomic Radiation) committee of the U.S. National Academy of Sciences. With the committee finally selected in their favor, the geneticists pushed their agenda through with a recommendation that a linearity at low dose assumption be adopted for estimating reproductive risks in humans from exposure to ionizing radiation. Their argument was not based on data that could adequately address this question, far from it, but rather on an unproven dose–response hypothesis set within a context of societal fear and perceived global responsibility. This unified group of geneticists believed that only they had the necessary insights into the mutational effects of radiation; it was their solemn responsibility to protect future generations. They therefore pushed this agenda forward based on a protectionist philosophy, such as today's precautionary principle concept that their ideological offspring promote, even though it lacked the scientific basis to make their case.

Muller had a strong interest in the nature of the dose–response for radiation-induced mutation. Soon after he had observed that X-rays could cause mutations in reproductive cells, he directed studies to determine the shape of the radiation-induced dose–response curve. Muller guided two researchers, Clarence P. Oliver (1898–1991), later to became a professor of genetics at the University of Minnesota and later still at the University of Texas at Austin and Fred B. Hanson (1886–1945), later to become the associate director of the Natural Sciences Division of The Rockefeller Foundation, in his laboratory, to better address whether the mutational dose–response was linear. Similar research was also initiated by a number of other investigators in the immediate aftermath of Muller's seminal findings. However, the results that emerged did not experimentally resolve the issue of the shape of the mutagenicity dose–response [48]. In fact, the lowest dose tested was still strikingly high, being 275 rads, a truly massive dose to the fruit fly's gonads. This dose was comparable to receiving well over

1,000 chest X-rays in 3.5 min! In addition, most of the published attempts to demonstrate linearity during this time period failed to do so, giving further support to the threshold dose–response concept. Despite very limited data, a lack of overall consistent findings, and the fact that low doses were never even remotely assessed, Muller nevertheless inexplicably developed a very firm, although incorrect, public conviction that mutation frequency is directly proportional to the dosage absorbed, with no evidence of a threshold dosage below which the treatment is too dilute to work [65].

Based on the findings of Oliver and Hansen, which supported an X-ray–induced linearity interpretation even though their exposures were grossly excessive, Muller soon showed his inclination to extrapolate X-ray–induced mutation findings in a linear manner. In what may well have been the very first such effort in foreshadowing the future field of risk assessment, he tried to estimate the background spontaneous mutation rate in fruit flies from ionizing radiation using the linearity method. When his predictions were wrong by approximately 1,300-fold [69], Muller was forced to reassess the significance of background radiation, yet his flirtation with the linearity at a low-dose relationship would remain.

According to Carlson [65], Muller displayed this same belief nearly a decade later in his report to the Medical Research Council of Great Britain. In this report Muller [70] suggested that no exposure to ionizing radiation existed below which mutations could not occur. Therefore, regardless of how much the dose may be attenuated as a result of its dilution in environmental media, ionizing radiation posed a mutagenic risk.

That Muller continued to strongly adhere to this public belief in the linearity hypothesis may be seen in his acceptance speech for the Nobel Prize in December of 1946, which affirmed that Oliver, Hanson, and Temofeeff had shown that the frequency of gene mutations is definitely proportional to dose, despite the extremely high cumulative doses and dose rates used. If one doubted his definitive position on linearity, he then cited the research of former student Ray-Chaudhuri [71,72] which, according to Muller [67], leaves "no escape from the conclusion that there is no threshold." Muller's unequivocal Nobel Prize Lecture conclusion apparently was not shared by Ernst Caspari. In a letter to Curt Stern, Caspari stated that the difficulty with the Ray-Chaudhuri [73] data involved confusion over the appropriateness of the control group and that the experimental error was quite large. The Ray-Chaudhuri study was of very modest size, failed to include numerous important methodological details, failed to include critical data on lethal clusters, the sterility and fertility of the females, sex ratios, and the age of the males, among others. Of further note is that he changed to a different fruit fly strain halfway through his study without explanation. This new strain had a control group mutation rate of only one third of the previous strain, yet the data of both strains were combined with the author claiming there were no differences between the strains. Despite these and other misgivings, the Ray-Chaudhuri research lowered the dose rate to 0.01 rad/min for a continuous exposure of 43,200 min (30 d). The result was a cumulative dose to the fruit flies of 400 rads, an exposure that was approximately one fifth that which demonstrated approximately five times as much damage. Such findings supported the linearity interpretation. However, the 400-rads/30-day exposure to the flies would exceed human background rates (cosmic radiation and local gamma radiation) by many thousandfold. Less than two years after Muller asserted the no escape phrase, data from Caspari and Stern [74] suggested that linearity was not observed in the fruit flies at the lowest dose rate yet tested.

That the Caspari and Stern [74] findings directly challenged the linearity assertions was especially interesting because Muller was a paid consultant to Curt Stern on this project, even supplying the fruit flies [68]. In fact, Stern had sent a draft of the Caspari and Stern manuscript to Muller. In a letter to Stern, dated November 12, 1946 (a month before his December 12, 1946 Nobel Prize Lecture), Muller acknowledged the data challenging his linearity perspective, their potential significance, and the urgent need to replicate the study [75]. Despite his knowledge of these data, which were far more substantial, much better documented, and used approximately one sixth the dose rate of the Ray-Chaudhuri experiments, Muller delivered his linearity pronouncement as if it were unassailable; his real message should have been that more study was needed to resolve this issue [76].

A key question then was how the findings of Caspari and Stern [74] could be marginalized without adversely affecting the careers and reputations of these two well-known geneticists. This would also have to be done within a framework that did not expose Muller's Stockholm deception. This was achieved in a two-step process [77].

The first involved making the discussion of the Caspari and Stern manuscript somehow disavow their findings without finding fault with the data. Caspari and Stern [74], with the encouragement of Muller [75], achieved this goal by arguing that their threshold supporting data could not be accepted until it was determined why their findings differed from that reported in Spencer and Stern [78]. This study also assessed the effects of ionizing radiation on the frequency of sex-linked recessive lethal mutations in the germ cells of fruit flies. However, it was a study with numerous important differences from that of Caspari and Stern [74]. For example, the Spencer study treated the flies with X-rays, not gamma rays, gave their cumulative dose (50 rads) acutely, that is, over only 2 min, whereas the same cumulative dose required constant exposure for 21 d for Caspari. The diets used by the two studies were totally different, markedly affecting the percent sterility and other reproductive parameters. In all, at least 20 significant differences between the studies made them impossible to compare directly, making the demands of their discussion unrealistic and foolish even to propose. Yet Caspari and Stern [74] demanded that the scientific community not accept their findings until they determined why the two studies reported differing mutation rates. They did not apply that constraint to the Spencer and Stern study. In his January 14, 1947 letter to Stern [79], Muller indicated that it would be acceptable to publish the Caspari paper because now so many qualifying statements appeared (i.e., "cautions"); this was most likely because it would not hurt the linearity case [75]. Because Stern was then the editor-in-chief of *Genetics,* their manuscript would get published even with its inappropriate and misdirected discussion.

The next step was to complete the replication study. This chore was given to a new master's student by the name of Delta Emma Uphoff. The problem was that Uphoff was new to *Drosophila* research, lacking the experience and expertise of Spencer and Caspari, both of whom were exceptionally talented and experienced, being the equal of Stern himself. In her replication of Caspari, the control group was aberrantly low. This resulted in Uphoff and Stern rejecting their findings, saying they were uninterpretable [80]. In fact, in a very unusual course of action, Stern apparently forced Uphoff to note in the discussion that part of the problem may have been bias on behalf of

the "experimenter" (Delta Uphoff, presumably). A second experiment by Uphoff also displayed an aberrantly low control group, again making the findings useless. The third and final experiment seemed to work, because they reported that a dose of radiation that was double that used by Caspari had induced a significant increase in the germ cell mutation rate. This led to the summarized publication of all the findings, including those of Spencer and Caspari in a brief (slightly more than one page) technical note in *Science*. In this paper, Uphoff and Stern [80] concluded that no dose existed below which radiation could not induce a mutation, a major conclusion, in a major journal, by an eminent geneticist, the editor of the most influential genetics journal. This conclusion would carry considerable weight.

Of particular concern is that these authors failed to point out that their significant mutational findings in their third and final experiment gave evidence of being aberrantly high, being nearly threefold greater than would have been predicted even by a linear model. Of considerable importance is that Uphoff and Stern [80] promised to provide the scientific community with the documentation to support their conclusions but they never did. Thus, Uphoff and Stern [80] provided three new experiments, each with aberrant findings and none of the promised documentation. Despite these critical flaws, acceptance of the Uphoff and Stern [80] perspective was rapid and widespread, as reflected in historical perspectives by leaders in the mutagenicity field [81,82]. The conclusions of the Stern research team were especially highlighted in the profoundly influential publication by future Nobel laureate E.B. Lewis [83] when he made his case for ionizing radiation linearity to be extended to cancer induction as well. According to Neel [81], this linearity conclusion even landed Stern a term (1950–1953) on the Advisory Committee to the Division of Biology and Medicine of the Atomic Energy Commission during a critical period in which health policy relating to radiation research was being formulated, all setting the stage for the BEAR I Committee.

Although the genetics community accepted Uphoff and Stern's [80] undocumented conclusion of linearity for ionizing radiation–induced germ cell mutation in the fruit fly, they were also focused on newly undertaken research at the University of Rochester by Donald Charles and at the Oak Ridge National Laboratory by William Russell with the mouse model. The case of the University of Rochester mouse studies had important problems, mostly centering around Charles, who frustrated his colleagues by providing only unofficial draft assessments that he continued to revise but failed to finalize [68]. The best that Charles did was to publish a 3.5-page partial summary, lacking any presentation of research methods, of his extensive radiation mouse studies in the journal *Radiology* in 1950 [84]. Unfortunately, Charles died of leukemia in 1955, never publishing any further account during the critical lead up to the BEAR I Committee activities. In 1961 some former colleagues attempted to summarize the study results, but only in a very limited fashion [85].

In the case of the mega-mouse type studies of Russell, they too were unable to provide significant scientific insight during the years leading up to the recommendations of the BEAR I committee. During this pre-1956 period, Russell's experimental studies dealt with high doses, with the lowest dose being 300 rads. In fact, the Atomic Energy Commission Special Ad Hoc Advisory Committee on Genetics had recommended that he lower the dose to 150 and even 75 rads. According to Jolly [68], Russell was determined not to go lower than 150 rads because of the insensitivity of his model. The committee further stressed the need to push in the direction of lower doses, perhaps with other more sensitive models, to determine whether the nature of the radiation-induced mutation dose–response was linear or threshold. According to Jolly [68], even though the genetics community knew that no convincing direct evidence existed of linearity at low dose for radiation-induced mutation at this time, they nevertheless were committed to the perspective that the mutagenic effects of ionizing radiation were linear, cumulative, and deleterious. This was the genetics community mind-set as led by Muller and his colleagues as the BEAR I committee began their historic deliberations (November 1955 to June 1956). It was also a mind-set that, according to James Crow [86], a member of the BEAR I Committee, was guided by principles that were "mostly from *Drosophila* research."

What Muller really believed on the dose–response issue may be gleaned from a 1949 letter to Robley Evans (1907–1995), an MIT professor critical of the low-dose linearity hypothesis. Muller stated that "many of the quantities are only very roughly known even for *Drosophila*, and we are admittedly extrapolating, it is all we can do in our present state of ignorance and we must meanwhile remain on the safe side" [65].

While the genetics community was consolidating its belief in linearity at low dose for the radiation-induced mutation concept, dissenters arose strong enough to resist the group perspective. For example, Willard Ralph Singleton (1900–1982) (trained by L. J. Stadler [1896–1954] at the University of Missouri), at the Brookhaven National Laboratory, was an outspoken critic of the linearity at low-dose mutagenicity hypothesis. His research revealed a nonlinear relationship between mutation rate and dose rate, with disproportionate increases in mutations occurring as the dose increased and nonmutagenic responses at lower doses, thereby challenging the belief in linearity at low dose [87–89]. During the deliberation period of the BEAR I committee, *The New York Times* (April 17, 1955) published an article that provided the opportunity to challenge the emerging linearity at low dose consensus for germ cell mutagenicity. In that article Singleton stated: "there is probably a safe level of radiation below which no genetic changes occur." Jolly [68] noted that even though Singleton was a well-accomplished genetics researcher, his findings and interpretations were, for the most part, ignored because they were in conflict with the emerging and soon-to-be-dominant linearity paradigm.

The significant uncertainty of the nature of the mutagenicity dose–response in the low dose zone and the uncertainty of extrapolating results from fruit flies to humans had little impact on Muller and his geneticist colleagues. Their linear dose–response recommendation received the authority of the National Academy of Sciences, and the United States was on its way to rejecting the threshold dose–response in favor of the linearity at low dose model for the assessment of radiation-induced reproductive damage. Within approximately a year's time, the focus shifted to somatic effects and the National Committee for Radiation Protection, following the recommendation of E.B. Lewis (1918–2004), one of Muller's geneticist colleagues and former student of C.P. Oliver, pushed through the first-ever formal recommendation that radiation-induced cancer also be assumed to act via linearity at low doses. Again, the data did not support the case for a cancer linearity argument. In fact, the case that Lewis [83] had made was considered laughable (see Table 2, page 212 of Calabrese [3]), not even requiring a response by opposing leaders in the field [90]. However, which person/idea wins is often determined by who is in power, as seen in the garnering of influential editorial support from Grahame P.

DuShane (1910–1963), the editor-in-chief of *Science* [91], along with major favorable stories in *Life* magazine (June 10, 1957) and other powerful outlets. Within a few years, multiple national and international advisory committees copied the lead of the National Committee for Radiation Protection, and their low-dose linearity recommendation soon became national policy and remains so even today [48].

The action of BEAR I was a major moment in U.S. regulatory history that was quietly achieved, yet with stupendous consequences. It became the official dogma of U.S. regulatory agencies.

The issue of linearity at low dose for radiation-induced cancer was occurring during the later part of the 1950s. Ironically, the Delaney Amendment to the 1958 Food Additives Amendment in the U.S. became law on April 26, 1958 (http://en.wikipedia.org/wiki/Delaney_clause). It stated "no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal." This Delaney clause was later inserted into the Color Additives Amendment of 1960, following the Thanksgiving cranberry crisis of 1959 due to the presence of the herbicide aminotriabole, an animal carcinogen, in cranberries [92,93]. Despite their parallelism in time, there was no apparent interaction between the development of a linearity at low dose methodology for radiation-induced cancer and the science and political framework employed to support the decision to prevent adding carcinogens to food.

During this period, James Delaney (1901–1987), a member of the House of Representatives from the state of New York, began to interact with Dr. Wilhelm Hueper, a National Cancer Institute scientist, and a leading expert on environmental and industrial carcinogens. Hueper offered a very strong protectionist philosophy to Delaney along with powerful credentials, thereby allowing Delaney to proceed. Because scientists were unable to define what a safe level of exposure to carcinogens may be, along with not understanding their mechanisms of action, Delaney asserted that no risk was worth taking with respect to chemical carcinogens, and that chemicals did not have rights.

In the case of radiation, a different concept of risk evolved that related to permissible risk that could be estimated with the linear model. The Delaney amendment, inspired by the strong views of Hueper, was to lead to the prevention of possible exposures. The Food and Drug Administration would later modify the Delaney amendment to address the concept of a de minimis risk, so that carcinogens could be added to the food supply if they were estimated to have a risk less than a certain value (for example, one in a million/lifetime), following a linearity at low dose model. Thus, in time, the radiation and food additive risk perspectives converged. Committee 17 of the Environmental Mutagen Society attempted to have the Delaney Amendment generalized to include chemical mutagens in the early 1970s, but failed to achieve this goal, falling back to the earlier guidance of the 1956 BEAR I committee that assessed genetic risks within the context of a doubling dose framework that was still consistent with the linearity at low dose model [94,95].

National Academy of Sciences–Safe Drinking Water Committee

Nearly 20 years later, the first National Academy of Sciences (NAS) Safe Drinking Water Committee (SDWC) [96] adopted the linear at low dose risk assessment compromise of the late 1950s, somewhat updated, and applied it to chemical carcinogens. Their actions make as little sense today as they should have in 1977. The NAS SDWC failed to provide an adequate evaluation and set of recommendations on this risk assessment issue. In their document, eight guiding principles for the support of low-dose linearity for cancer risk assessment were discerned. Within approximately two decades, six were shown to be untenable, another impossible to study practically, and the eighth had yet to be demonstrated [48] (see Table 4, page 217 in Calabrese [48]) (Appendix 2). Their highly precautionary oriented recommendation was presented to the U.S. EPA in the 1977 publication entitled *Drinking Water and Health*. Their eight principles (ie, assumptions) had emerged from a new generation of unified geneticists of the early 1970s as summarized in published comments during a high-level genotoxicity conference chaired by Alexander Hollaender (1898–1986) with the proceedings in *Environmental Health Perspectives* (see the detailed set of comments after the Ernst Freese (1925–1990) paper [97]; these comments present the views of multiple genetic toxicologists concerning mutation and dose–response).

Further confounding this faux pas was another apparent miss by the NAS SDWC. While the Committee was drafting their rubber stamp-like statement on chemical carcinogens and linearity at low doses, other researchers had published a major new finding that could have redirected the committee on the dose–response issue for carcinogens. A March 1977 paper in *Nature* by Samson and Cairns [98] revealed for the first time that a low dose of a chemical mutagen induced an adaptive response that led to protection against a subsequent and more massive exposure to that same mutagen. The paper ushered in the field of adaptive response and its widespread generality. If this paper had been read by the committee or its staff, it might have changed the course of cancer risk assessment for chemical carcinogens. However, the *Nature* paper was never cited in *Drinking Water and Health*.

Not surprisingly, the U.S. EPA accepted the linearity at low dose recommendation of the Committee and applied it to trihalomethanes (chloroform and related agents) by 1979 and then to a subsequent very long list of other chemical carcinogens for the rest of the century to the present. Life in the world of cancer risk assessment has not been the same since. Thus, even though a plethora of new studies have been published challenging the linearity at low dose perspective for cancer, the die was cast based upon an anemic, at best, assessment of the NAS SDWC, an assessment that missed one of the more significant and relevant new findings.

The NAS brings together some of the best and the most experienced professionals in a no conflict of interest manner in which biases are attempted to be balanced. This is the way it is described in writing, giving assurances of objectivity and scientific integrity to Congress, the scientific community, the media, and the public. However, two NAS committees failed on the most critical questions of the past half century. The first failure (the BEAR I committee) came from the geneticist community that introduced ideology into risk assessment. What this committee achieved was a dramatic failure of process, demonstrating that its ends justified the means, setting an unacceptable precedent. In the case of the next generation of NAS experts, the Safe Drinking Water Committee simply became enveloped by the ideologically oriented perspectives of their geneticist and biostatistical colleagues. It was a committee that accepted a series of assumptions without proposing how to validate the dose–response model they promoted into long-standing regulatory influence.

All we are saying... is give validation a chance

While the threshold model was being restricted to the assessment of only noncancer endpoints, the FDA decided to determine the nature of the dose–response in the low dose zone for genotoxic carcinogens. To achieve this goal, the FDA undertook the largest rodent study ever, using some 24,000 female mice (BALB/c strain), the so-called mega-mouse/ED01 study. A single carcinogen was tested, 2 acetylaminofluorene (2-AAF), known to be a mutagen and to cause tumors at multiple sites in different animal models, but especially in the bladder and liver of females of this mouse strain.

It was thought that much would hinge on the findings of this study, including whether the United States would base its carcinogen risk assessment methods on using a linear at low dose model or an alternative. So substantial were the scientific and societal implications that the U.S. Society of Toxicology created a 14-member expert panel to provide an assessment. Their analysis led to the publication of nearly an entire issue of the Society's journal, *Fundamental and Applied Toxicology* [99]. What they found surprised everyone. The dose-time-response was strikingly hormetic for the bladder cancer endpoint, occurring in each of the six different rooms housing the animals. In effect all six replications of the study agreed. The formal writeup by the expert Society of Toxicology panel strongly emphasized the J-shaped dose–response with beneficial effects at low doses as seen in their quoted comments from page 77: "The most striking aspect...is the reduction in probability of bladder cancer from control to doses 30, 35, and 45 ppm. This reduction occurs in all six rooms and is statistically significant... the ED01 study provides more than evidence of a 'threshold.' It provides statistically significant evidence how low doses of a carcinogen are beneficial" [99]. Thus, in the largest rodent cancer study ever undertaken, the data revealed a hormetic response. Despite these striking findings, the U.S. regulatory agencies failed to modify their approaches to carcinogen risk assessment policy and practice.

The foray of U.S. regulatory agencies into dose–response model validation for carcinogen responses using a standard rodent experimental model did not confirm the linearity at low dose hypothesis for bladder cancer nor even the threshold dose–response model. Huge amounts of money were spent, expectations were high, and in the end the federal agencies would not follow the data. The process was expensive, prolonged, and traumatic, all factors that would probably prevent any similar bureaucratic risk taking in the foreseeable future.

Failure of the threshold dose–response model

In the course of developing a methodology to assess the possible validation or limitations of the hormesis model, the question arose as to whether the threshold dose–response model had ever been validated. The general assumption was that it must have been validated, because it was now nearly 70 years since this model had been accepted and integrated into the lexicon of mainstream pharmacology and toxicology. Search we did, using every conceivable database and spectrum of relevant search terms and their combinations, along with the assistance of science librarians trained to uncover difficult-to-find entities. We simply could not find any attempt that had ever been published to assess the capacity of the threshold dose–response to make accurate predictions in the low dose zone, that is, below the threshold. Having reached the proverbial dead-end, we undertook our own attempt to validate the capacity of the threshold dose–response model to make accurate predictions in the low dose zone.

The validation study of the threshold dose–response was initially undertaken from a data set created from the pharmacological and toxicological literature using rigorous a priori entry and evaluative criteria. These criteria were applied to all the published studies in three journals (*Environmental Pollution*, *Bulletin of Environmental Contamination and Toxicology*, and *Life Sciences*) from the time of their inception in the mid 1960s to the present. The threshold dose–response model predicts that responses below the threshold should vary or bounce in a quantitatively similar fashion on either side of the control, just like random noise in a system. If the threshold model is dominant, then the ratio of responses above and below the control value should be very close to 1. The surprise was that the ratio did not approach 1; it exceeded this value by approximately 250%, a frequency that is far beyond any reasonable probability. The threshold model was not able to account for the findings in the below threshold zone. However, the responses below the threshold did display a consistent pattern, very closely paralleling the hormesis model [100,101].

This study had two major conclusions. The first indicated that the threshold dose–response model failed to make accurate predictions in the below threshold zone. The data set was intentionally very general, including data from plants, microbes, invertebrates and vertebrates, and from a wide range of biological endpoints and chemical agents, thereby enhancing the significance of the findings. Second, the validation study strongly supported an hormetic interpretation, findings that were consistent with the thousands of hormetic dose–responses that had been previously assessed based on a priori evaluative criteria.

This new study was especially important because the rules of the game applied equally to the threshold and hormetic models. The hormetic model could no longer be ignored. These were findings as important as they were unexpected.

Publicity follows hormesis

The validation data were important because they challenged a 70-year-old dose–response tradition. Our manuscript [100] was submitted to the journal *Toxicological Sciences,* the main journal of the U.S. Society of Toxicology, and made it successfully through their typically thorough peer-review process. Soon the toxicological world would learn that the threshold model was not as good as they had long been taught, whereas the hormetic model had performed far better than they might have imagined. In fact, we thought that this paper was one that had the potential to alter the toxicological landscape.

At approximately the time that *Toxicological Sciences* accepted our manuscript, I received a letter from the editor-in-chief of *Nature* with an invitation to write an article on hormesis. This publication in *Nature* provided a significant boost for the hormesis concept [102]. The key decision was its placement in the journal's media package, a position of high visibility. Soon we were inundated with calls for interviews from leading publications and other media outlets all over the world. Articles on hormesis quickly appeared in *The Wall Street Journal* [103], *Forbes* [104], *Fortune* [105], *Discover* [106], *Scientific American* [107], *Science News* [108], *Insight* [109], *Reason Online* [110], *U.S. News and World Report* [111], and in major stories in large daily newspapers like *The London Times Online* [112], *St. Louis Post-Dispatch* [113], *Boston Globe* [114], *The Baltimore Sun* [115], and others. Of further note

was that *Science* [116] published a four-page story on hormesis. Hormesis had finally arrived and with a big splash.

As a result of the *Toxicological Sciences* and *Nature* papers, interest in the hormesis concept was on a marked upswing. Round two of the validation tests would also take place in the journal *Toxicological Sciences,* using a large National Cancer Institute public database (57,000 dose–responses) that assessed the effects of nearly 2,200 potential anti-tumor drugs on 13 different strains of yeast, 12 of which had a different genetic error similar to some found in human cancers. Following the same basic plan as before, the dose–responses were put through a rigorous set of a priori entry and evaluative criteria and multiple statistical evaluations [117]. Regardless of the statistical analysis strategy, the findings once again strongly supported a hormetic interpretation whereas the threshold dose–response model performed extremely poorly, in effect, failing the test. Round three in the validation series was a study using yet a different approach; this time an *Escherichia coli* strain was tested in over 2,100 different potential antibiotics within an experimental replication framework. The results were the same, good job of predicting below threshold results for the hormetic dose–response model, but a poor job for the threshold model [118].

The threshold dose–response had now failed a third major challenge, strongly undercutting the scientific status of the U.S. EPA and FDA's default model used to establish most health standards. How many times would the threshold model have to be shown to be inadequate before regulatory agencies would reconsider their continuing acceptance of it as their gold standard, that is, the default model?

Linearity model performance: Noncancer endpoints

Of potential importance in the head-to-head comparisons between the threshold and hormetic dose–response models was that the predictive capacity of the linear at low dose model was also tested. It was not the main focus of the studies, because the endpoints were noncancer. However, in each of the extensive validation studies, the linear at low dose model was a failure, just like the threshold dose–response model. These observations challenge the general predictive utility of a linear at low dose model. Despite these findings, a recent NAS committee has proposed [119] generalizing the linearity at low dose model to all endpoints. Although this position was principally hypothetical, our data demonstrate that it fails to predict accurately in the low dose zone, even more than the threshold model.

Big pharma-enhancing biological performance and hormesis

In contrast to governmental regulatory agencies, which can be affected by ideological perspectives, businesses follow data that lead to profit. The early decades of the 20th century witnessed pharmaceutical companies racing to discover agents that would destroy major disease- causing microbes. The late 1940s likewise revealed the birth of cancer chemotherapy with its massive expansion in the following decades. Although killing cancer cells and harmful organisms has been a major pharmaceutical preoccupation, the 1970s ushered in a new initiative for this industry, one that concerns enhancing biological performance, all kinds of performance. These include, but are not limited to, improving memory, strengthening bone, enhancing sexual performance, growing more hair, faster and stronger wound healing, reducing anxiety, and reducing the risks of seizures. In each of these cases, the increased performance was attributable to the hormetic dose–response, all with copious supportive pharmacological studies [120]. The pharmaceutical industry and their regulatory oversight agency, the FDA, are at the core of these discoveries and their implementation within society. Not once, however, has the industry or the FDA credited or linked such successes to hormesis.

Pharmaceutically oriented scientists have called these performance-enhancing drug–induced dose–responses by a wide range of names, including biphasic, diphasic, parabolic, bitonic, bell-shaped, U-shaped, J-shaped, inverted U, low dose stimulation, pre-conditioning and several others, but not hormesis. In this process, the research community has failed to recognize that these biphasic dose–responses, which affect so many different types of biological endpoints, display the same quantitative features. These responses are not a haphazard grouping of dose–response entities that exhibit such remarkable similarity by chance. In fact, the quantitative features of the hormetic dose–response are the same regardless of the biological system studied, whether at the cell, organ, or individual level, the endpoint measured, or the chemical inducing the effect. It displays remarkable generality. Of considerable potential importance is that the hormetic dose–response likely provides a quantitative index of the limits of biological plasticity for each of these drug-induced performance enhancing effects [121]. In so doing, the hormesis concept reveals the magnitude of drug-induced responses that pharmaceutical companies can expect in human populations, and whether developing their product further would be profitable. This knowledge can be a key determinant in designing preclinical and clinical studies and could have a major impact on the assessment of clinical efficacy. The industry has unfortunately failed to adequately appreciate that hormesis is a broadly integrative and central biological principle that can revolutionize the drug development process. Nonetheless, in its own way, this industry has embraced the hormesis concept, in principle, in practice, and in fact. They have yet to embrace it by name. The failure of the pharmaceutical industry to use the term *hormesis* reflects its origin in traditional medicine and the long-standing conflict with homeopathy. This conflict has now come full circle. The dose–response explanatory principle of Schulz, so strongly rejected by the medical community nearly a century ago, underlies much of the success of the modern pharmaceutical industry in an ironic twist of scientific fate and promises to be even more significant in the future.

Final perspectives

The nature of the dose–response and its underlying mechanisms will remain toxicology's raison d'etre. The central issue of toxicology has quickly transformed into that of a low dose paradigm to reflect the societal concerns in which most people live. The capacity to investigate low doses has been revolutionized with respect to profound advances in chemical analysis, which has been directly linked to experimental systems for in vitro studies in which large numbers of concentrations of chemicals can be studied. In fact, the resurgence of interest in hormesis is being driven by such technical improvements, with a focus on assessing the biological effects of chemical and physical agents at low doses. Although high dose toxicology is not yet a historical remnant, and may never fully be so, the present and future of toxicology are in the low dose domain. This powerful development will drive the field for the foreseeable future and places hormesis directly at the forefront.

During the entire decade of the 1980s, only 10 to 15 citations per year could be found of the terms *hormesis* or *hormetic* in the vast Web of Science database. In 2010 alone, the number of citations exceeded 3,200, a sign of growing acceptance and

progressive integration within the scientific community and its research foundations. Its success and influence emerge from its broad generality across biological models, endpoints measured, and chemical and physical stressor agents along with its potential biomedical significance and reproducibility.

The hormesis concept has become integrated into a growing number of highly influential textbooks [122–124], and its usage by biomedical scientists has expanded worldwide. Five monographs on hormesis have also been published within the past few years [125–129]. Hormesis has also become a central concept in the areas of aging and biogerontology as well as becoming a foundation for pharmaceutical agents designed to improve biological performance, especially in the areas of anxiety reduction, memory enhancement, stroke damage prevention, bone strengthening, wound healing, skin care, the numerous domains of pre- and post-conditioning and other areas. These developments point to expansive growth, enhanced clinical significance, and biomedical centrality.

While this brief summary strongly suggests that the future will be hormesis-oriented and central to the biomedical community, several identifiable institutional factors preserve the dose–response status quo. Each is regulatory-agency oriented and reflects specific manifestations of the historical dose–response strategy of traditional medicine during the mid decades of the 20th century, as detailed earlier. These dose–response impediments include the following: research funding by governmental regulatory agencies is likely to ignore hormesis, the fear that hormesis will weaken environmental exposure standards, the default model in risk assessment is a vehicle for conservative risk estimates, and the U.S. EPA's definition of a risk assessment denies the capacity to incorporate health benefits.

Research funding by governmental regulatory agencies

Federal funding agencies control the direction of much research in the United States and elsewhere, including who and what gets funded, as well as the language and culture of research. This process directly affects what ideas and data get published, read, and believed. Broadly diversified funding sources both within and outside of government are an important means to broaden research goals and directions as well as to enhance the likelihood that valid research ideas, including those dealing with hormetic hypotheses, are not minimized or excluded because of historical, structural, or ideological biases. For example, because the U.S. EPA definition of a risk assessment explicitly excludes the concept of a beneficial or adaptive response, it suggests that the Agency would fail to prioritize and therefore be less likely or even fail to fund hormetic hypotheses relating to such beneficial responses (See *Responses excluded in U.S. EPA risk assessment*). A certain proportion of regulatory agency research funding should be made independent of regulatory agency control via the use of external panels to enhance the objectivity of the granting process, from the development of the research priorities to the awarding of the specific grants.

Fear that hormesis will weaken exposure standards

Although the hormetic stimulatory response has the capacity to induce both beneficial and adverse health effects depending on the specific biological context [130], various authors have incorrectly truncated this definition to only include a beneficial effect. This has led to the further position that the hormesis concept will undercut many of the environmental gains that have been made over the past four decades, eventually resulting in weakened environmental health standards. In fact, the opposite is likely to be the case. An hormesis-guided risk assessment process provides decision-makers with the most complete dose–response information on the biological/toxicological effects of the agent tested, especially in the low dose zone. By long ignoring the hormetic/biphasic dose–response, the biomedical community and regulatory agencies failed to discern the occurrence of endocrine disruption effects at doses below the traditional toxicological threshold [120]. In a similar fashion, oncologists have also long missed the capacity of numerous anti-tumor drugs to enhance the proliferation of tumor cells in patients because of their longstanding belief in threshold dose–response model predictions [131]. Thus, by incorporating the hormesis concept into the risk assessment framework, including the design of the bioassay, risk estimates would be more confidently based regardless of whether the hormetic hypothesis was supported by the data, and whether low-dose stimulatory responses were harmful or beneficial.

The default model in risk assessment

An important issue for regulatory agencies such as the U.S. EPA is what dose–response model is selected as the default in risk assessment. A default dose–response model is usually selected when insufficient data are available in the standard bioassay to convincingly identify the best-fitting dose–response model. This situation occurs nearly all the time in practice. In these cases, the agency will routinely default to the most conservative model. This situation creates a self-fulfilling prophesy of model selection because the poorly designed standard chronic bioassay (that is, too few and too high doses) does not permit one to distinguish in a statistical manner amongst the dose–response models. As noted, several large-scale dose–response validation studies have indicated that the threshold and linear at low dose models do not make reliably accurate predictions in the low dose zone, whereas the hormetic model does. Also, in many thousands of other studies the dose–responses fail to reflect threshold or linear responses while conforming to the hormetic model. Yet these poorly performing dose–response models are guaranteed to win the default model contest for regulated chemicals because of the design limitations of the standard chronic bioassay. The U.S. EPA therefore uses a chronic bioassay that inevitably leads to the selection of a default model that fails in validation studies! Therein lies the self-fulfilling highly conservative risk assessment prophecy of the status quo, which regulatory agencies have failed to correct.

Responses excluded in U.S. EPA risk assessment

The U.S. EPA [132] risk assessment goal is to prevent pollutant-induced harm while not considering possible health benefits (i.e. "as the purpose of a risk assessment is to identify risk [harm, adverse effects, etc.], effects that appear to be adaptive, non-adverse or beneficial may not be mentioned"). This goal creates a framework in which the hormetic dose–response could be ignored. For example, this suggests that the EPA would consider possible harm related to an hormetic/biphasic dose–response, but not if benefits occurred. The U.S. EPA could accept data showing a low dose hormetic stimulation leading to adverse health effects (e.g., increased prostate gland size [120] or significant acceleration in a developmental process such as the onset of puberty [133,134]). In contrast, the U.S. EPA would ignore the hormetic response when it resulted in a reduction in a population-based risk (reduction in tumor incidence).

This discussion illustrates that the U.S. EPA concept of risk is too limited, because two types of risks are present (risk of harm and risk of losing a benefit). Both need to be considered when providing an integrated public health assessment. This position was strongly supported in a recent survey of the membership of the U.S. Society of Toxicology and the Society of Risk Analysis in which 68% advocated for the incorporation of health benefits into the risk assessment process [135].

The U.S. EPA risk assessment policy statement is also inconsistent with community-based programs for fluoride. Fluoride risk assessments have historically centered on preventing harm at high doses while being flexible enough to ensure the existence of community-based drinking water fluoridation programs at lower doses, that is, a beneficial response.

Another conflict of this policy occurs when an agent displays a beneficial effect for one segment of the population at a dose that would be harmful to another population-based subgroup (for example, a high-risk group). In similar situations, the same agent may display a beneficial effect with the high-risk group at a low dose, while having no measurable biological effect on the normal segment of the population at this dose. This general set of conditions/possibilities would be expected to be a common occurrence.

The above series of policy-based inconsistencies indicate that the U.S. EPA risk assessment policy guidance document is problematic by failing to consider interindividual variability (different stakeholder groups) in a hormesis-based risk assessment process. Therefore, the benefits and risks to each of these groups would have to be considered, quantified, made explicit in the assessment process, and then integrated within a comprehensive negotiation or risk management decision. Although this more comprehensive methodology presents new challenges for agencies such as the U.S. EPA and individual states, it also reflects emerging biological realities that must be dealt with and managed, all with the goal of estimating an optimized population-based response.

The 2004 U.S. EPA risk assessment guidance document [132] fails to integrate the health assessment needs of society. Its limited definition of a risk assessment creates a significant gap in the assessment of human health, leaving an institutional blind spot. This flawed U.S. EPA strategy will lead to inadequate population-based health assessments and wasteful allocation of resources. I suggest a revision to the U.S. EPA definition of a risk assessment to one that estimates the net population-based toxicity incidence at each level of incremental exposure.

How will this dose–response debate turn out? The area of the biological effects of low dose exposures will proceed at an expanding pace independent of the regulatory agencies. This will have a transforming impact on understanding of the nature of the dose–response in the low dose zone and its underlying mechanisms as well as a plethora of new public health and biomedical implications. The real issue is to what extent regulatory agencies will embrace such research and incorporate its findings within their risk assessment paradigms. Unless such changes occur within regulatory agencies, society might see an interesting, though troubling, dichotomy in which the world of pharmaceuticals and health care products become based to an ever greater extent on the hormesis concept, improving the quality of our lives, while environmental regulatory agencies cling to an anachronistic belief/policy that their mission is only to prevent harm and not to optimize public health.

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Appendix 1A. Quotes relating to homeopathy and the Arndt-Schulz Law [18].

High dilution scheme of Hahnemann lacking credibility – page 24

"Hahnemann, for example, claimed that drugs at the 30th potency produced reliable effects, and actions produced by similar dilutions are still occasionally described (e.g. Konig, 1927). A homeopathic potency means a dilution of hundredfold, and hence the 30th potency corresponds to a concentration of 1 part in 10^{60}. This works out at about one molecule in a sphere with a circumference equal to the orbit of Venus. Such results may be either believed or disbelieved, but their acceptance involves discarding the fundamental laws of chemistry and physics."

"Other results have been published which are almost equally improbable."

Associates Schulz with homeopathy – page 195

"In 1885 Rudolf Arndt put forward the suggestion that if a weak stimulus excites an organism, then any drug in sufficiently weak dose ought to do this also. This suggestion was developed by Schulz, who had a leaning to homeopathy."

Challenges the biological significance of the Arndt-Schulz Law – pages 195-196

"...many pharmacologists have pointed out that it (Arndt-Schulz Law) expresses no general truth. It is interesting to note that no trace of evidence in support of such a law can be found in the majority of drugs."

The Arndt-Schulz Law probably confused with experimental errors – page 196

"As in the case of potential actions, evidence in favor of this law can easily be obtained from experimental errors."

Appendix 1B. More quotes on homeopathy and the Arndt-Schulz Law [19].

Challenges mechanistic understanding of the Arndt-Schulz Law effects – page 215

"...laws have been enunciated which merely state that certain phenomena frequently occur, without providing any explanation or their occurrence. The Arndt-Schulz Law...(is) (an) example of this type..."

Arndt-Schulz Law is usually discredited when carefully assessed – page 204

"Arndt-Schulz Law. This law states that any drug which causes stimulation at low concentrations will cause inhibition at high concentrations. This law is in accordance with homeopathic doctrines and hence has maintained a certain popularity. The law is true in so far that nearly all drugs if given in sufficiently high dosage or concentration will produce injury or death in living cells."

"The chief objection to the law is that it is obviously untrue in the case of most drugs that have been studied carefully."

"Many of the effects which appear to support this law have found simple explanations..."

Arndt-Schulz Law was related to vitalism – page 30/example 1

"Diphasic actions of drugs on tissues are frequently observed, and their occurrence led to the postulation of the Arndt-Schulz Law, which states that drugs which paralyze at high concentrations stimulate at low concentrations. It is true that such effects are often observed but there is no necessity to postulate any mysterious (emphasis added) property of living tissues because similar effects are frequently observed with enzyme systems."

Arndt-Schulz Law was related to vitalism – page 30/example 2

"This peculiar effect is mentioned here because it is the simplest example known to the writer of a reaction following the "Arndt-Schulz Law". In this case a high concentration of oxygen prevents the formation of HbCO but if hemoglobin is exposed to a low concentration of carbon monoxide, then a low concentration of oxygen may increase the formation of HbCO. Hence oxygen may be said to stimulate in low concentrations and to inhibit in high concentrations. This diphasic action can be explained on physico-chemical grounds and although our present knowledge is inadequate to explain most of the diphasic actions met with in more complex systems, yet there seems no reason to consider them as peculiarly mysterious (emphasis added)."

Challenges high dilution proposal of Hahnemann and homeopathy – page 26

"...Hahnemann claimed that drugs produced effects when given in the 30^{th} potency...in the case of a drug with a molecular weight of 100, (this) corresponds to 1 molecule in about 100,000 liters. It is obvious that (when) a sample of a few c.c. of such a mixture is taken, the odds against the presence in the sample of a single molecule of the drug are at least a million to one. Hence the claims of the homeopathist conflict more immediately with the laws of mathematics, physics and chemistry than with the biological sciences. It does not appear necessary for pharmacologists to discuss the evidence adduced by the homeopathists until the latter have succeeded in convincing the physicists that they have demonstrated the existence of a new form of subdivision of matter. It may be mentioned that the existence of such recognized subdivisions of the atom as electrons, etc. does not help the homeopathic claims in a significant manner because, to explain the results of Hahnemann, it is necessary to assume that a molecule can be divided into millions of sub-units."

Appendix 2. National Academy of Sciences Safe Drinking Water Committee (1977) low dose linearity guiding principles: no longer tenable three decades later [48].

Only one or two changes in a cell could transform it and this could lead to cancer.	Not tenable
Human population heterogeneity was a factor, and some people may be at greater risk. Such heterogeneity leads to the conclusion that there was no population-based threshold.	Impossible to practically study
A transformed cell will be irreversibly propagated.	Not tenable
If the mechanism involved mutation, there would be no threshold; in fact, if there were no information on mechanism and cancer occurred, mutation should be assumed.	Not tenable
It is necessary to assume that a single molecule or a few molecules can cause a mutation. Therefore, linearity at low dose can be assumed.	Not tenable
There is also the assumption that the exposure would be directly additive to background, if acting via the same mechanism. This would also support the linearity conclusion.	Generally not shown
Available mutagenicity data with radiation indicated that it was linear at relatively low doses.	Not tenable
Since chemical carcinogens act like ionizing radiation, low dose linearity should also be assumed to be the case for such chemicals.	Not tenable

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Review

Cancer risk assessment: Optimizing human health through linear dose–response models

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ABSTRACT

This paper proposes that generic cancer risk assessments be based on the integration of the Linear Non-Threshold (LNT) and hormetic dose–responses since optimal hormetic beneficial responses are estimated to occur at the dose associated with a 10^{-4} risk level based on the use of a LNT model as applied to animal cancer studies. The adoption of the 10^{-4} risk estimate provides a theoretical and practical integration of two competing risk assessment models whose predictions cannot be validated in human population studies or with standard chronic animal bioassay data. This model-integration reveals both substantial protection of the population from cancer effects (i.e. functional utility of the LNT model) while offering the possibility of significant reductions in cancer incidence should the hormetic dose–response model predictions be correct. The dose yielding the 10^{-4} cancer risk therefore yields the optimized toxicologically based "regulatory sweet spot".

1. Introduction

The assessment of cancer risks from exposure to ionizing radiation and chemical carcinogens by regulatory agencies worldwide is typically performed via the use of linear at low dose modeling. The linear non-threshold (LNT) approach for cancer risk assessment was first proposed for cancer risk assessment by the U.S. National Committee for Radiation Protection and Measurement (NCRPM) in 1958, following the recommendation of the U.S. National Academy of Sciences (NAS) Biological Effects of Atomic Radiation (BEAR) I Genetics Panel to switch from a threshold to a linear model for assessing genomic risk from ionizing radiation in 1956 (Jolly, 2003; Whitemore, 1986).

The LNT approach was later adopted by regulatory agencies starting in the late 1970s assessing risks for chemical carcinogens in all media (e.g. air, water, food and soil) (National Academy of Sciences (NAS), 1977). The initial transition from the threshold to the LNT approach in the mid 1950s was made prior to the discovery of DNA repair, adaptive responses with chemical mutagens and ionizing radiation, apoptosis, pre-conditioning and the resurgence of the hormetic concept, all of which could affect the shape of the dose response in the low-dose zone. The clarification of different mechanisms of action for carcinogens has encouraged the development of cancer risk assessment methods that incorporate knowledge of species specificity and threshold. These approaches are often employed by the U.S. EPA and FDA and most European authorities for non-genotoxic carcinogens (Page et al., 1997; Whysner and Williams, 1992; Williams, 2001; Williams et al., 2012).

These developments have challenged the theoretical and mechanistic basis of the LNT, along with the recognition that epidemiological methods are in effect not capable of detecting risks below twice the normal background (Taubes, 1995). Furthermore, the massive mega-mouse study that used 24,000 animals was only able to estimate risk at the 1% level (ED01 study) (Bruce et al., 1981). Similar limitations were reported for a cancer bioassay study with >40,000 trout (Bailey et al., 2009). These methodological limitations along with the more recent developmental insights on the plethora of adaptive mechanisms that act at low doses have revealed limitations of the LNT model.

2. Developments

The dose–response model that has been shown to have biological plausibility, especially in the low dose zone, is hormesis, a biphasic dose–response. Current interest in hormesis can be traced back to the research of Thomas Luckey on radiation hormesis (Luckey, 1980) and on chemical hormesis by Tony Stebbing (Stebbing, 1982). These researchers stimulated the electric power utilities of Japan

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and the U.S. to conduct the first hormesis conference in August, 1985. These three events reactivated interest in the hormesis concept.

Since the initial hormesis conference mentioned here, multiple books have been published on hormesis (Calabrese, 1992, 1994; Costantini, 2014; Elliott, 2008; Luckey, 1992; Mattson and Calabrese, 2010; Rattan and LeBourg, 2014; Sanders, 2010; Stebbing, 2011). Also, many chapters on hormesis in toxicology and pharmacology texts have been produced; hormesis has been the focus of more than a dozen conferences; multiple symposia at major society meetings have addressed hormesis. It is the subject of more than 2000 scientific publications in peer-reviewed journals, and the object of more than 30,000 citations in the Web of Science/Knowledge. Extensive documentations of hormetic dose responses have been summarized from a large and continuously updated database (Calabrese and Blain, 2005, 2009, 2011).

The hormetic dose–response was also found to make more accurate predictions than the LNT or threshold dose–response models in head-to-head comparisons using large, independent data sets (Calabrese and Baldwin, 2003; Calabrese et al., 2006, 2008). Detailed mechanisms of 400 hormetic dose responses have recently been summarized (Calabrese, 2013). Additionally, the hormetic dose response therefore has been demonstrated to be highly generalizable, being independent of biological model (i.e., phylogenetically diverse – from bacteria to humans; in vitro and in vivo), level of biological organization (i.e., cell, organ and organism), endpoint, inducing agent and mechanism.

3. Objective – Integration

Based on these features, it has been proposed that the hormetic dose–response should become the default model for risk assessment for both carcinogens and non-carcinogens. The hormesis database provides strong evidence that dose–response relationships for carcinogens (e.g., DDT, dioxin, multiple PAHs, ionizing radiation) and non-carcinogens typically display hormetic dose response patterns with similar quantitative features. While this line of argument has been made (Calabrese, 2004), this is not the purpose of this paper. The present paper proposes a "practical" and straightforward harmonization of both the LNT and hormetic models for cancer risk assessment. As is customary in such convergences, common ground is sought by various entities (e.g., regulatory agencies and regulated industries), while differences are still recognized and will remain unresolved for now.

We see the following reasons why integration of both models would be beneficial. First, if hormesis describes low-dose exposure impacts of chemicals/ionizing radiation more accurately than the LNT-model does, then the regulatory authorities should apply the best that the toxicological sciences have to offer. The hormetic dose response requires rigorous study designs in order to be properly evaluated, with large numbers of doses, with proper dose spacing, and often within a dose–time framework. When such data are available, the hormetic dose response has far outperformed the threshold and linearity dose response model for accuracy in estimating low dose effects (Calabrese and Baldwin, 2003; Calabrese et al., 2006, 2008).

Second, considering the developments in analytical chemistry, increasingly lower levels of chemicals can be detected. We have entered the realm of atto- (part per quintillion; 10^{-18}) and zeptomoles (part per sextillion; 10^{-21}) of detectable analytes (Pagnotti et al., 2011). Consequently, the unspoken 'logic' of the LNT-model infers that a 'clean bill of health' can never be truly given (Hanekamp et al., 2012). The technology-driven stringency of regulation in the context of the LNT-model can be attenuated with the aid of the biphasic dose–response model. As a result, regulatory expenditures will be reduced along with benefit optimization (Keeney, 1997).

Third, the biphasic dose–response model underscores the beneficial adaptability of organisms' responses to chemical exposure, whereby regulation that expresses the functional integration of both the LNT and hormetic models is better able to address society's fears of carcinogen exposure.

4. Integration – Roadmap

How then do we envision this integration, that is, the harmonization of the hormesis and LNT dose response models for cancer risk assessment? The reconciliation of these two divergent models can surprisingly be made in a direct and uncomplicated fashion.

1) The key aspect of the hormesis/LNT convergence is that when risks are based on chronic animal bioassay studies, the optimal protective effects (i.e., reduction in tumor incidence for the affected below the control group) is predicted to occur at the same dose at which the LNT predicts 10^{-4} risk.
2) To achieve this value, the hormetic-based approach would first estimate a 1% response from the animal bioassay via a BMD-type methodology. When this derived-dose is divided by factor of 100, it yields slightly less than a risk of 10^{-4}. This was shown to be the case for ten highly diverse data sets by Gaylor (1989). The hormetic risk assessment methodology of Calabrese and Cook (2005), which is optimized at the same dose that the LNT estimates a 10^{-4} risk level, predicts benefit while the LNT estimates enhanced cancer risk.
3) We propose that cancer risk assessment adopt an acceptable risk of 10^{-4} using the LNT model since this dose would also yield the optimal hormesis dose response benefit. This dose is the so-called regulatory "sweet-spot" that provides substantial protection against theoretical low dose risks that are far below the detection of even the most demanding epidemiological and toxicological studies/methods, while including benefits predicted by the hormetic dose response model (Fig. 1). This approach would also have the significant societal benefit of affecting a profound reduction in costs (i.e., financial and predicted adverse health), markedly affecting cost/benefit analyses.
4) In a population of one million people, the 10^{-4} risk predicts 100 people (i.e., 10^6 people $\times$ 10^{-4} risk = 100) affected with an organ-specific cancer (e.g., lung, kidney, bladder, etc.) by some deleterious agent that is added to the background for cancer of that organ (Fig. 1). Assuming a 25% tumor background

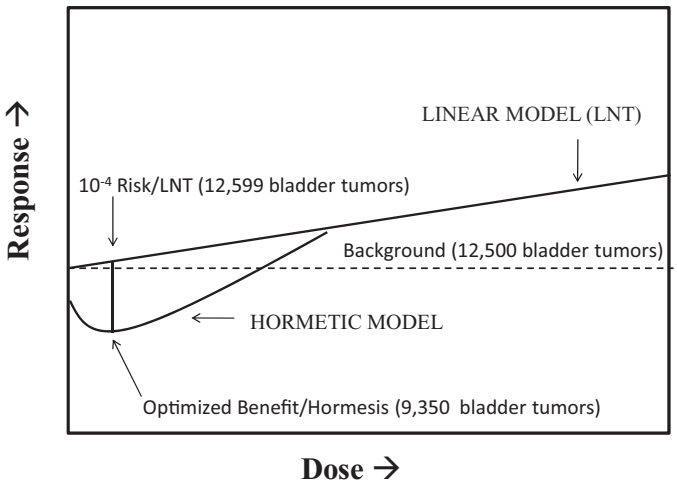

Fig. 1. Functional integration of hormesis and LNT for carcinogen risk assessment; derivation of the optimal regulatory strategy.

incidence, 250,000 of the one million people would be predicted to develop tumors. If the organ in question was responsible for 5% (e.g., bladder) of the above 25% (i.e., 250,000 people), it would represent 12,500 of the 250,000 people with cancer (i.e., $0.25 \times 0.05 = 0.0125$) ($0.0125 \times 10^6 = 12{,}500$). Many organ-specific tumors, including the bladder, affect about 3.5 to 6.0% of the tumor occurrence (National Cancer Institute (NCI), 2014), thus the use of 5% for an organ like the bladder would be a reasonable expectation. Organs affecting a notably higher proportion of people (e.g., about 16–18% per cancer type) are those cancers of the lung, breast and prostate. The 100 newly affected people with *chemically* induced bladder cancer are then randomly distributed among the entire population of one million. This suggests that 25% of the 100 will already be in the process of developing a background tumor, with about 5% of those already targeted for a "spontaneous" bladder tumor ($0.25 \times 0.05 = 1.25\%$). The net result of background (i.e. spontaneous) and tumor-induction via a chemical carcinogen at 10^{-4} is 12,500 ("background") plus 100 new chemically induced cases (i.e., $12{,}500 + 100 = 12{,}600$) minus 1 due to spontaneous and induced bladder tumors in the same individuals. This would yield a total of 12,599 individuals with bladder cancer. The hormetic benefit is likely to affect both background and induced tumor incidence, reducing their incidence by roughly 25% (Calabrese and Blain, 2011), lowering the predicted total number of affected people (12,500) by about 3150. There can be other situations in which the chemical may affect multiple organs with different tumor backgrounds and induced tumor incidence, affecting the nature and complexity of the assessment. For example, in the case of dioxin, it was shown in the Kociba et al. study (Kociba et al., 1978) that has been widely used for cancer risk assessment that hormetic effects appear to occur in multiple organs (i.e., Females: liver, ovary, uterus, cervix/vagina, mammary, pituitary and adrenal; Males: liver, pulmonary, pituitary, pancreas and adrenal). In such cases it may be possible to select that dose which displays the lowest overall tumor incidence for risk assessment purposes. In theory, this type of situation may be predicted to have a greater beneficial effect than described for the bladder cancer. However, it would not be unexpected for the optimal effect to vary by organ. Using a financial metaphor, the convergence of the LNT/10^{-4} risk and hormesis methodologies permits the protection of one's "principle" (i.e., impossible to detect chemically-induced increase in cancer risk) while adding considerable benefit (i.e., large reduction in cancer risk for those affected organs). This compromise strikes an optimized balance in which there is a very low theoretical risk increase and a very high theoretical benefit. Choosing a 10^{-6} acceptable risk would reduce 99 of the 100 theoretically affected people while eliminating the possible hormetic benefit. This type of strategy would prevent the possibility of beneficial effects, which could be substantial.

5) The example presented above addresses the risk of a single complete carcinogen. However, humans live in a highly complex environment involving exposure to a vast array of complete carcinogens, tumor promoters, chemoprotective chemicals and physical agents, all superimposed on dynamic metabolic processes, numerous adaptive mechanisms and complex exposure dynamics. Predicting cancer incidence of complex mixtures from experimental and epidemiological studies is problematic, if not impossible. A very limited, simplified and yet mechanistically oriented approach to assess complex carcinogenic mixtures is the toxic equivalent factor (TEF) that assumes additive processes that act identically (e.g. same receptor) for similarly grouped agents (e.g. dioxins, PAHs and PCBs). The TEF concept was integrated within a LNT perspective. Epidemiological evaluations of complex mixtures reveal the failure of predictions of animal studies to predict human responses. For example, a cup of coffee contains >1000 chemicals of which approximately 30 have been tested for cancer. Of these the majority were carcinogenic in standard rodent model testing. Each cup of coffee contains >10 mg of rodent carcinogens, with American adults drinking three cups per day (Ames and Gold, 2000; Gold et al., 1992). The situation gets more complex as more carcinogens are added via the roasting process. However, despite such exposures to natural and roasted process-related carcinogens, comprehensive epidemiological studies reveal neutral or beneficial effects from lifetime coffee drinking depending on the organ (Bohn et al., 2014; Crippa et al., 2014). Thirty-two occupational epidemiological studies (i.e. case–control – 19 studies; cohort – 13 studies) of gasoline exposure which is a highly complex and variable mixture of >500 saturated/unsaturated hydrocarbons revealed no pattern or clear association between gasoline and any cancer (Keenan et al., 2010). Furthermore, dose responses of complex mixtures [e.g. petroleum (Laughlin et al., 1981), waste-water treatment effluents (De Nicola et al., 2004; Mendoza-Figueroa, 1973; Walsh et al., 1982), complex organochlorine mixtures (Aube et al., 2011)] over a broad dose response often conform to an hormetic dose response. These findings support the conclusion that complex mixtures can induce hormetic dose responses and can be evaluated within the framework proposed here.

6) An important implication of model uncertainty is that it has the potential to undermine and challenge the use of LNT in toxic tort litigation cases. The acknowledgement of substantial and unresolved uncertainty in risk assessment may preclude causation judgments with low dose exposures. In fact, the use of LNT in toxic tort cases in the United States has been successfully challenged in numerous litigations affecting ionizing radiation, asbestos as well as chemical carcinogens, principally due to its lack of validation capacity, inconsistency with published findings and the recognition of substantial adaptive mechanisms that undermine an LNT interpretation (Milward v. Acuity Specialty Products Groups, Inc, 2013; Sutera v. Perrier Group of America Inc, 1997; Whiting v. Boston Edison Co, 1995).

5. Discussion

The search for public health common ground via the integration of opposing risk assessment models is a new approach in the process of risk assessment harmonization. It permits the strengths of opposing perspectives to be incorporated into a unified risk assessment approach. It is recognized that estimates of low risk is a speculative activity, especially when the data are derived from high dose toxicology studies and that there is no current practical way around this limitation. The present recommendation is viewed as substantially conservative, creating the opportunity to benefit from the induction of adaptive responses while recognizing and incorporating model uncertainty into the risk assessment process. We believe that this is a sound foundation upon which to base environmental public health policy.

The precautionary principle, which is at the core of modern governmental environmental health policies, is founded on a toxicological assumption that lower is always safer/better and that zero exposure, especially for carcinogens, is the goal [maximum contaminant level goal (MCLg)] as seen for EPA drinking water standards. The precautionary principle was strongly influenced during its formative development by belief in LNT predictions. Harmonizing of the LNT and hormesis dose response models can provide a vehicle not only for cancer risk assessment but also a novel means, along

with a more biologically based foundation, to guide a broad range of precautionary principle applications.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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The hormesis database: The occurrence of hormetic dose responses in the toxicological literature

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ABSTRACT

In 2005 we published an assessment of dose responses that satisfied *a priori* evaluative criteria for inclusion within the relational retrieval hormesis database (Calabrese and Blain, 2005). The database included information on study characteristics (e.g., biological model, gender, age and other relevant aspects, number of doses, dose distribution/range, quantitative features of the dose response, temporal features/repeat measures, and physical/chemical properties of the agents). The 2005 article covered information for about 5000 dose responses; the present article has been expanded to cover approximately 9000 dose responses. This assessment extends and strengthens the conclusion of the 2005 paper that the hormesis concept is broadly generalizable, being independent of biological model, endpoint measured and chemical class/physical agent. It also confirmed the definable quantitative features of hormetic dose responses in which the strong majority of dose responses display maximum stimulation less than twice that of the control group and a stimulatory width that is within approximately 10–20-fold of the estimated toxicological or pharmacological threshold. The remarkable consistency of the quantitative features of the hormetic dose response suggests that hormesis may provide an estimate of biological plasticity that is broadly generalized across plant, microbial and animal (invertebrate and vertebrate) models.

1. Introduction

Hormesis is a dose–response phenomenon in which opposite effects are observed at low, compared to high, doses for the same measured parameter. This will result in either an inverted U-shaped or a J-shaped dose–response curve. The concept of hormesis has received considerable interest in the toxicological, pharmacological and general biomedical areas over the past 10–15 years (Calabrese, 2008; Calabrese and Baldwin, 2001a, 2003b). For example, in the entire decade of the 1980s the Web of Science database reported about 10–15 citations per year for the terms hormesis or hormetic. However, in 2010 alone the number of citations was 3269 with publications in over 100 journals covering a broad range of biomedical disciplines. In 1997 Calabrese and Baldwin (1997a) reported on the creation of a relational retrieval hormesis database along with *a priori* entry criteria and numerous study parameters on which data would be entered. In 2005, Calabrese and Blain published the results of a detailed assessment of the database which contained nearly 5000 dose responses (Calabrese and Blain, 2005). The findings indicated that hormetic dose responses were observed in a broad range of biological models (i.e., from plant to human), occurring over a diverse set of biological endpoints and across a wide range of chemical classes. The analysis also revealed that the stimulation in the low-dose zone was typically modest with the maximum stimulation being generally 30–60%. The overall findings were important since they demonstrated that hormetic dose responses were reproducible and broadly generalized. Several related publications extended these findings, providing a frequency estimate of hormesis within the toxicological and pharmacological literature (Calabrese and Baldwin, 2001b, 2003a; Calabrese et al., 2006, 2008). These studies also revealed that the hormetic dose response was far more common than the threshold and linear dose response models in direct comparisons using the same *a priori* entry and evaluative criteria. The present paper extends the 2005 study of Calabrese and Blain by presenting an updated analysis of the hormesis database that has approximately 9000 dose responses. The updated analysis strengthens the basic findings of the original paper (Calabrese and Blain, 2005) with respect to the conclusion that hormesis is highly generalized with no apparent restriction to biological model, endpoint, or chemical classes.

1.1. Database entry criteria

The hormesis database was created using a specific set of *a priori* criteria designed to:

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(1) identify probable cases of hormesis,
(2) assess the quantitative features of the hormetic dose response, and
(3) assess the generalizability of the hormetic phenomenon according to biological model, endpoint, and chemical class and physical stressor agents.

The hormesis database inclusion criteria have been previously published (Calabrese and Blain, 2005) and will only be discussed briefly. They include: (1) a minimum of 10% stimulation (i.e., inverted U-shaped dose response) or a 3% depression of response (i.e., J-shaped dose response). (2) If the 10% or 3% selection criteria were not satisfied, a dose response could have been entered into the database if the response achieved statistical significance in hypothesis testing. (3) The study had to employ an adequate concurrent control for comparison. As a general rule, dose responses that satisfied the above three criteria, satisfied hypothesis testing requirement, and provided the most detailed assessment of response both above (toxic zone) and below (hormetic stimulatory zone) the threshold were prioritized for selection into the database. Such dose responses offered the best opportunity to provide more detailed quantitative assessment of the broader dose response continuum, as well as a more robust assessment of overall dose responses.

1.2. Database scoring methodology

Each dose response entered into the hormesis database was scored according to Calabrese and Baldwin (1997a,b) and Calabrese and Blain (2005) in which numerical values were awarded based on the quality of the study design, response magnitude, statistical analysis, and reproducibility of the response in order to evaluate the capacity of an experiment for demonstrating hormesis (i.e., strength of evidence). Points were awarded for (1) the number of doses below the zero equivalent point (ZEP; location where response crosses the control value), (2) estimation of a ZEP (i.e., curve crosses the control value or was such that the curve would estimate where it would cross the threshold), (3) the number of statistically significant responses below the ZEP, (4) the magnitude (percent of control value) of the stimulatory response, and (5) reproducibility of the data by other studies with data provided (in other publications or within the same publication). The points are summed and the evidence of hormesis is awarded as indicated in Table 1.

This scoring methodology rewarded studies that explicitly considered below zero equivalent point (ZEP) or NO(A)EL doses in their study designs with the balance of the points being skewed in favor of response over design. While only a single point is awarded for dose responses that identify or could estimate a ZEP, the points for response progressively increase from one for a minimal entry starting at 10% (or 3%) up to four points for 400% (or 0%) of the control. Because increases of 400% or greater than the control may represent a different phenomenon than hormesis, the points were arbitrarily capped at four for responses 400% of the control or greater. Statistical significance was also emphasized in the scoring since hypothesis testing considers the sample size, variability, and magnitude of response in a reliable and nonbiased manner.

1.3. Description of the database

The hormesis database contains 2527 citations with 8962 dose responses (i.e., endpoints). The articles have been obtained mainly through extensive searches through numerous journals,

Table 1
Summary of criteria with assigned point values used in the quantitative evaluation of hormesis.

Doses below ZEP (n)	Point value	ZEP determined/estimated	Point value
Study design criteria			
1	1	Yes	1
2	2	No	0
3	3	–	–
4	4	–	–
≥5	5	–	–
Doses statistically significant (n)	**Point value**	**Reproducibility**	**Point value**
Response criteria			
1	2	Yes	3
2	4	No	0
3	8	–	–
≥4	16	–	–
Inverted U-shaped curve	**J-shaped curve**		**Point value[a]**
Magnitude of response (percentage control value)			
≥110% ⩽ 125%	⩽97% ⩾ 92%		0.5
>125% ⩽ 150%	<92% ⩾ 84%		1
>150% ⩽ 200%	<84% ⩾ 68%		2
>200% ⩽ 400%	<68% ⩾ 5%		3
>400%	<5%		4
Total point range		**Hormesis evidence category**	
Summary of total point ranges			
1–2		No–low	
>2–8		Low	
>8–12		Low–moderate	
>12–16		Moderate	
>16–20		Moderate–high	
>20		High	

[a] The point value is multiplied by the number of experimental doses falling within the corresponding percentage range. For example, if an experiment has three doses exhibiting stimulatory responses within the 125–150% range with the curve approaching the ZEP and two of the responses achieve statistical significance and one does not, the total number of points would be: 3 × 1 = 3 (Doses below the ZEP; study design criteria); 1 (estimated ZEP; study design criteria); 4 (response criteria, statistically significant); 3 × 1 = 3 (magnitude of response), for a total of 11 points. The 11 points would achieve the categorization of hormesis evidence of "low–moderate".

Table 2
Articles in the hormesis database by year of publication.

Publication year	Number of articles	Percent of total (2527)
<1930	39	1.5
1930–1939	30	1
1940–1949	45	2
1950–1959	95	4
1960–1969	158	6
1970–1979	429	17
1980–1989	505	20
1990–1999	706	28
2000–2010	520	20.5

cross-referencing journal citations, MEDLINE, Web of Science and other database searches using multiple words such as hormesis, hormetic stimulation, inverted U-shaped, J-shaped, biphasic, bell-shaped, and others. The articles have occurred in a diverse array of journal publications (nearly 500 different journals), although several books (51 citations), theses and dissertations are included. Information on the citation, chemical, biological model, study duration, treatment, endpoint, and dose–response curve are entered into the database (see Calabrese and Blain, 2005 for specific entry fields). Queries can be conducted using any of the fields. The query system was employed to yield the descriptive assessments offered in this article.

1.4. General

The database is arranged so that each citation is associated with studies (i.e., experiments). Data on gender and different species are considered separate studies as are different variations in study design. Each study may have examined several endpoints (e.g., body weight, survival). Each endpoint has a dose response associated with it. Any search performed with the database provides the number and percent of total for citations, studies, and endpoints. The database includes experimental findings from 1899 to the present (Table 2). However, the majority (85%) of the articles in the database were published after 1970.

Reproducibility was difficult to implement because of the uncertainty over what constitutes a bona fide case. It was decided that reproducibility should only be claimed in cases where the follow-up study was essentially identical to the original study. This would explain why only nine citations (with a total of 15 responses) were determined to be reproducible between publications. Reproducibility reported within the same article occurred with 258 dose responses in 133 different articles. However, a dose response is only considered reproducible when the article provided the results of the separate experiments. Cases where data were combined and reported as averages or where the study author claimed that the results were reproducible and only the results from a representative study were provided were not considered reproducible in the database due to lack of data for confirmation.

2. Study design considerations

2.1. Agent

Nearly 2000 different agents from approximately 245 different chemical classes have been entered into the database, about twice as many as reported in 2005. While 81% (i.e., 7216) of the 8962 dose responses entered into the database used a chemical agent, 19% (i.e., 1746) employed radiation or radioactive material. Table 3 provides the chemical classes and physical agents with the greatest frequency in the database. Calabrese and Blain (2004) have examined the hormetic response of metals in greater detail elsewhere.

2.2. Model

In the 2005 publication, plants and animals were equally represented in the database. However, in the last 5 years more animal studies have been entered with animal models predominating

Table 3
Most prevalent chemical classes and physical agents in the hormesis database.

Chemical class/physical agents	Number of dose responses	% of total (8962)
Inorganics (including elements and metals)	1717	19
Radiation/radionuclides	1746	19
Organics	5499	62
Organophosphate/pesticides/herbicides/fungicides	573	6
Hormones/amino acids/fatty acids/enzymes/protein/neurotransmitters/neuropeptides/peptides/steroids	425	5
Alcohols/phenols	410	5
Carboxylic acids	349	4
Heterocyclic compounds	233	3
Chlorinated hydrocarbons/halogenated hydrocarbons/chlorinated furanone	202	2
Organometals	171	2
Nitrate/nitrile/nitro compounds/nitrofurans/nitrosamines/nitroso compounds/nitrosoureas	163	2
Hydrocarbons/PAHs/aromatic hydrocarbons	152	2
Amides/amines/imides/imines	143	2
Antibiotics/antifungals/antivirals/antiprotozoals/antiseptics	132	1.5
PCB/PBB	102	1
Antineoplasics	89	1
Azo compounds/azo dyes/ azoic dye fragment/dye intermediates	89	1
Plant extracts/alkaloids/alkaloid derivatives/pyrrolizines alkaloids	79	1
Dioxins	73	1
Flavanols/Flavones/flavanones/flavonoids	67	1
Carbamates	59	1
Polymer/polyamine/polynucleotide/polysaccaride	55	1
Aldehydes	47	0.5
Ester/ethers	42	0.5
Mycotoxins	38	0.4
Sulfonic acids	35	0.4
Miscellaneous[a]	631	7

[a] Miscellaneous chemical class refers to complex chemicals or chemicals that could not be placed in a specific chemical class.

Table 4
Experimental models by year of publication and test system.

Year of publication	Experimental model								
	Animal			Plant			Bacteria		
	Total	In vitro	In vivo	Total	In vitro	In vivo	Total	In vitro	In vivo
Before 1970	323 (7%)[a]	104 (32%)[b]	219 (68%)	981 (31%)	350 (36%)	631 (64%)	151 (19%)	142 (94%)	9 (6%)[c]
1970–1979	604 (12%)	252 (42%)	352 (58%)	912 (29%)	150 (16%)	762 (84%)	78 (10%)	78 (100%)	–
1980–1989	1119 (23%)	568 (51%)	551 (49%)	451 (14%)	131 (29%)	320 (71%)	352 (44%)	352 (100%)	–
1990–1999	1639 (34%)	763 (47%)	876 (53%)	452 (46%)	139 (31%)	313 (69%)	91 (11%)	88 (97%)	3 (3%)
2000–2010	1183 (24%)	823 (70%)	360 (30%)	370 (12%)	103 (28%)	267 (72%)	134 (17%)	132 (99%)	2 (1%)
Total	4868 (10%)	2510 (52%)	2358 (48%)	3166 (100%)	873 (28%)	2293 (72%)	806 (100%)	792 (98%)	14 (2%)

[a] Number in parentheses is the percent of the total row (e.g., 323/4868 = 7%).
[b] Number in parentheses is the percent for that year group (e.g., 104/323 = 32%).
[c] Bacteria experiments were considered in vivo when the bacteria was injected into a host (e.g., rat).

the database (54% animals as compared to 35% plants). Rats (1085 dose responses) and mice (1218 dose responses) followed by humans (794 dose responses) are the most commonly used animal models, which is similar to that observed in 2005. Although no particular plant species predominates wheat was the most common (188 dose responses). In addition, fungi (167 dose responses), algae (219 dose responses), and yeast (110 dose responses) were also broadly represented. Ninety-four (4%) of the citations compared the effects of an agent on a certain endpoint across different ages. The conclusion remains that hormesis occurs in all different developmental and age related stages.

Most of the dose responses in the database (i.e., 5889) either did not specify the gender studied or gender does not apply to the model (e.g., plants or cell lines). Males (2196 dose responses) and females (1804 dose responses) were used in approximately the same number of studies. Because males and females were used together in some in vivo studies but the results were not separated by sex, the total number of dose responses by sex will not equal the total number of dose responses in the database because the study will be counted under both male and female results. If results were separated by sex, they are reported as separate studies. The sexes were compared in 128 (5%) of the citations in the database.

2.3. Test system

In contrast to the 2005 publication, where in vivo experiments predominated, there are similar amounts of dose responses in the database conducted in vivo (4698; 52%) and in vitro (4284; 48%) in the present analysis. This may be due to the increase in animal studies conducted in vitro (Table 4). Animal models were conducted at a similar rate in vivo and in vitro, while plants were conducted more often in vivo (Table 4). Study durations varied greatly (i.e., from a few minutes to a few years) because of differences between in vivo and in vitro studies. Even in vivo studies can vary greatly from a single injection or a few seconds of radiation to a lifetime of repeated exposures. Because of the variety of methods employed, the type of control used also varied greatly; however, the control used was an adequate control to study the effect.

2.4. Time course

Because hormesis may be related to an adaptive response, it may only be observed at certain times after or during exposure. Studies that examine an effect over different time periods demonstrate that in many cases, the hormetic effect is observed only at certain time points, while in other cases the hormetic effect is consistent over the time points measured. There are 1324 dose responses in the database that examined an endpoint at more than a single time point. Only one of the repeated measurements is entered into the database. While 36% of the 1324 dose responses only had two measurements, 64% of the 1324 endpoints had three or more measurements obtained.

2.5. Hypothesis testing

A dose response was considered to have hypothesis testing if the study authors provided statistical results comparing the treatment group to the control. While only 45% of the dose responses had hypothesis testing that fit the criteria of the database, there were instances where statistical analysis was performed but the study authors did not provide comparisons between treatment and control. Mutagenicity studies have often used a twice above control value as an indication of a positive effect instead of hypothesis testing. However, mutagenicity studies had a similar use of hypothesis (47%; i.e., 422 of 892 dose responses) testing as the general database (45%). Table 5 demonstrates an increase in hypothesis testing after 1970 compared to before 1970 with the greatest use of hypothesis testing occurring in more recent years.

2.6. Transgenerational

The database includes 168 dose responses from studies that examined transgenerational effects. Each generation fitting the criteria is entered into the database as a separate study. There were 45 citations that examined generations indicating that more than one generation from a citation had a hormetic effect. Sometimes studies examined the transgenerational effects, but only reported results for the second generation and not the generation exposed. Therefore, the number of generations is considered only one in the database. This occurred in 5% of the dose responses, however, the majority (83%) of the studies examined two generations.

2.7. Subjects

The number of dose responses in which subjects were presented for inclusion in the database is 5576 (62% of the dose responses) (Table 6). Because some of the dose responses include a different number of subjects for different doses (e.g., controls had twice as many subjects or the endpoint was only measured in survivors and each group had a different number), the total (i.e., 5915) will not be equal to the number of dose responses (i.e., 5576) that included the number of subjects. The number of subjects was considered the number that the study authors provided in the tables or figures and could include the number of experiments, the number of cultures, or the number of individuals. Table 6 demonstrates that there are only slightly more in vivo studies than in vitro studies, but that in vivo studies were more likely to have >10 subjects per group.

Table 5
The number of dose responses with hypothesis testing by publication year.

Publication year	Number of dose responses	Number with hypothesis testing	Percent of yearly total
Before 1970	1470	212	14
1970–1979	1625	690	43
1980–1989	1950	729	37
1990–1999	2213	1308	59
2000–2010	1704	1136	67
Total	8962	4075	45

Table 6
Number of subjects per treatment group.

Number of subjects per treatment group	Number of dose responses	In vitro	In vivo
⩽10	3632 (61%)[a]	2165 (60%)[b]	1467 (40%)
11–50	1483 (25%)	297 (20%)	1186 (80%)
51–100	355 (6%)	74 (21%)	281 (79%)
101–1000	371 (10%)	68 (18%)	303 (82%)
>1000	74 (1%)	22 (30%)	52 (70%)
Total	5915 (100%)	2626 (44%)	3289 (56%)

[a] Number in parentheses is the percent of the total row (e.g., 3632/5915 = 61%).
[b]Number in parentheses is the percent for that number of subjects grouping (e.g., 2165/3632 = 60%).

Table 7
The number of dose responses by endpoint.

Endpoint type	Number of dose responses	Percent of total dose–response relationships (8962)	Plants (3166)	Animals (4868)	Bacteria (806)
Growth	3353	37	2197 (69%)[a]	922 (19%)	186 (23%)
Metabolic[b]	1996	22	598 (19%)	1157 (24%)	199 (25%)
Mutagenic[c]	892	10	51 (2%)	498 (10%)	342 (42%)
Immune response	581	6.5	–	581 (12%)	–
Survival	568	6	60 (2%)	415 (8.5%)	74 (9%)
Reproduction[d]	534	6	179 (6%)	342 (7%)	4 (0.5%)
Neurological	285	3	0 (0%)	285 (6%)	0 (0%)
Behavioral[e]	266	3	–	265 (5%)	–
Cancer	161	2	–	161 (3%)	–
Longevity	152	2	1 (0.03%)	148 (3%)	1 (0.1%)
Disease[f]	68	0.8	60 (2%)	8 (0.2%)	–
Damage[g]	61	0.7	19 (0.6%)	42 (0.9%)	–
Developmental[h]	45	0.5	1 (0.03%)	44 (0.9%)	–

[a] Number in parentheses is the percent of the total for that specific model (e.g., 2197/3166 = 69%).
[b] Examples: DNA repair, enzyme activity, hormone levels, ROS production, ATP response, oxygen uptake, or urine volume.
[c] Examples: number of revertants, micronucleus frequency, incidence of bent humeral bristles, chromosome aberrations, drug resistance, or DNA integrity.
[d] Examples: fecundity, hatching rate, eggs/female, number of young, number of resorptions, seed germination, or number of flowers.
[e] Examples: distance travelled, flinches/min, number of bites, rearings, or head dips, or number of correct choices.
[f] Disease in plants refers to rot or spoilage of fruit, diseased plants, or number of weeds; disease in animals refers to infection (e.g., kidney infection) or parasites.
[g] Damage in plants refers to disintegrating roots, decay, number of holes caused by insects or oxidation; damage in animals refers to cell rounding, ALT or LDH release, lesions, lipid peroxidation, or hyperplasia.
[h] Examples: adult eclosing rate or malformations.

2.8. Endpoints

The dose–response relationships are divided into two different sections for endpoint (endpoint type – e.g., growth; and endpoint parameter – e.g., body weight). Thirteen endpoint types were selected for generalized search capacity (Table 7). Since the 2005 publication, a neuroscience endpoint was added and the database was re-evaluated by endpoint parameter to add the neurological endpoint type. Although growth was the endpoint type associated with the most dose responses, there were numerous dose responses in each of the endpoint types. In addition, studies may examine multiple endpoint parameters that predict the same or closely related process (e.g., cell proliferation), but it is estimated via a different endpoint parameter (e.g., DNA synthesis, tritiated thymidine uptake, cell numbers, etc.) and each that meets the criteria is listed as a separate endpoint within the study. As is expected, the endpoint type measured the most frequently in plants was growth and mutagenic was the most frequently studied endpoint in bacteria. Animals had a more uniform distribution by endpoint type.

2.9. Hormetic curve

The majority of the dose–response relationships were inverted U curves (82%). Table 8 describes the width of the 5668 dose responses in which a range could be determined. The majority of the dose responses displayed a stimulatory response range less than 10-fold wide. However, the response range could be highly variable with a low percentage (7%) displaying a stimulatory range that exceeded 1000-fold. There was only a slight variation in the stimulatory range between the models with bacteria having a greater proportion of the dose responses having a stimulatory range between 10- and 100-fold (Table 8). Although stimulatory ranges of ⩾1000-fold occurred at the lowest frequency for all endpoints, this range occurred at a greater frequency in neurological and immune responses than the other endpoints (Table 9). The

Table 8
Width of stimulation range by model.

Width (-fold)	Number of dose–response relationships	Number in plants	Number in animals	Number in bacteria
⩾1 < 10	2450 (43)[a]	922 (54%)[b]	1290 (48%)	197 (35%)
⩾10 < 100	2054 (36)	572 (34%)	572 (21%)	233 (42%)
⩾100 < 1000	760 (13)	149 (9%)	484 (18%)	113 (20%)
⩾1000	404 (7)	58 (3%)	326 (12%)	18 (3%)
Total	5668[c]	1701 (100%)	2672 (100%)	561 (100%)

[a] The number in parentheses is the % of total dose–response relationships.
[b] The number in parentheses is the % of total for that model.
[c] The value of 5668 differs from the total number dose–response relationships (i.e., 8962) because calculation of the range was not possible for all dose responses for various reasons (e.g., data presentation precludes exact determination of range).

Table 9
Width of stimulatory range by endpoint.

Endpoint type	Number of dose responses with ranges available	Width of the stimulatory range			
		⩾1 < 10	⩾10 < 100	⩾100 < 1000	⩾1000
Growth	1971	1074 (54%)[a]	587 (30%)	201 (10%)	109 (6%)
Metabolic	1213	455 (38%)	435 (36%)	208 (17%)	115 9%)
Mutagenic	755	173 (23%)	402 (53%)	145 (19%)	35 (5%)
Immune response	304	100 (33%)	118 (39%)	51 (17%)	35 (11%)
Survival	465	277 (60%)	149 (32%)	32 (7%)	7 (2%)
Reproduction	313	163 (52%)	116 (37%)	24 (8%)	10 (3%)
Neurological	202	68 (34%)	58 (29%)	42 (21%)	34 (17%)
Behavioral	166	52 (31%)	82 (49%)	24 (14%)	8 (5%)
Cancer	75	18 (24%)	43 (57%)	9 (12%)	5 (7%)
Longevity	84	43 (51%)	36 (43%)	5 (6%)	0 (0%)
Disease	9	1 (11%)	5 (56%)	2 (22%)	1 (11%)
Damage	35	11 (31%)	16 (46%)	4 (11%)	4 (11%)
Developmental	37	15 (41%)	7 (19%)	13 (35%)	2 (5%)

[a] Number in parentheses is the percent of the total for that specific endpoint (e.g., 1074/1971 = 54%).

higher frequency of mutagenic endpoints with a stimulatory response between 10- and 100-fold supports the finding that bacteria were more likely to have a stimulatory range between 10- and 100-fold. Although the majority of dose responses had a stimulatory range less than 10-fold, there were some endpoint types (i.e., mutagenic, immune response, behavioral, cancer, disease, and damage) that had a greater frequency of dose responses with stimulatory ranges between 10- and 100-fold.

The relative proportions of maximum stimulatory responses for J-shaped curves were more evenly distributed than in inverted U-shaped curves (Table 10). The maximum stimulatory response range in inverted U-shaped curves was between 110% and 150% of the control; 79% of the dose responses had a maximum stimulatory response less than 200% of the control regardless of model or endpoint (Tables 10–12). The majority of J-shaped curves had a maximum stimulatory response between 50% and 100% of the control (Table 10). While this range is maintained regardless of the model employed (Table 10), certain endpoints had a greater tendency to have % of control values ⩽50% of the control (i.e., immune response, disease, and developmental) while other endpoints (i.e., survival and cancer) were more evenly distributed across the maximum stimulatory response ranges (Table 11).

Information on the distance from the maximum stimulatory response and the dose where the curve would cross the zero equivalence point (i.e., response equal to control; ZEP) is provided in Table 13. To calculate the point, the curve must have peaked and crossed the control value again. This occurred in 5331 (59%) of the dose responses. In the majority (62%) of cases the maximum stimulatory response is within a factor of five from the ZEP, which occurs regardless of biological model used (Table 13) or endpoint examined (Table 14). In plants, the majority of the dose responses are in vivo regardless of the distance from the maximum stimulatory response and the ZEP. In animals, however, as the distance between the maximum stimulatory response and the ZEP increased, the more likely the study was to be in vitro (Table 13).

2.10. Strength of evidence

Calabrese and Baldwin (1997a,b) provided a numerical scoring system to determine the strength of evidence for assessing to what extent the dose response was consistent with the hormetic dose–response model, which is described above in the scoring methodology section. Table 15 indicates that the majority of the responses (57%) had low evidence of hormesis. After entering nearly 9000 dose responses, this is more likely a limitation of the scoring system than in the quality of the dose responses. Although there are some dose responses with only one or two doses in the hormetic region, Table 16 indicates that there are as many curves with three, four, or five doses in the hormetic range. The low evidence results are due to a limited maximum stimulatory response in the predominantly inverted U-shaped curves and the lack of hypothesis testing in approximately 50% of the dose responses. In plants, the studies were generally conducted in vitro and this did not change with the number of doses below the ZEP. In animals, however, the in vivo studies were more likely to have fewer doses below the ZEP. As the number of doses below the ZEP increased, more of the animal studies were found to be in vitro (Table 16).

3. Discussion

Since the publication in 2005 3400 new dose responses have been added to the database, approximately 40% of the database (Calabrese and Blain, 2005). Despite the substantial entry enlargement of the database the quantitative aspects of the hormetic curve (i.e., maximum stimulatory response, stimulatory range,

Table 10
Maximum stimulatory response by model.

Maximum stimulatory response (% control)	Number of dose–response relationships	Number in plants	Number in animals	Number in bacteria
J-shaped curve				
⩽100 > 75	583 (37%)[a]	47 (26%)[b]	314 (30%)	219 (64%)
⩽75 > 50	526 (33%)	63 (35%)	372 (35%)	91 (27%)
⩽50 > 25	277 (18%)	32 (18%)	224 (21%)	21 (6%)
⩽25	193 (12%)	40 (22%)	144 (14%)	9 (3%)
Total	1579 (100%)	182 (100%)	1054 (100%)	340 (100%)
Inverted U-shaped curve				
⩾100 < 110	42 (0.6%)	11 (0.4%)	30 (0.8%)	1 (0.2%)
⩾110 < 150	4379 (59%)	1913 (64%)	2147 (56%)	253 (54%)
⩾150 < 200	1443 (20%)	572 (19%)	754 (20%)	98 (21%)
⩾200 < 500	1191 (16%)	397 (13%)	694 (18%)	73 (16%)
⩾500 < 1000	190 (3%)	62 (2%)	102 (3%)	22 (5%)
⩾1000	138 (2%)	29 (1%)	87 (2%)	19 (4%)
Total	7383 (100%)	2984 (100%)	3814 (100%)	466 (100%)

[a] The number in parentheses is the % of total dose–response relationships.
[b] The number in parentheses is the % of total for that model.

Table 11
Maximum stimulatory response in a J-shaped curve by endpoint.

Endpoint type	Number of dose responses with ranges available	Maximum stimulatory response			
		⩽100 > 75% of control	⩽75 > 50% of control	⩽50 > 25% of control	⩽25% of control
Growth	42	13 (31%)[a]	13 (31%)	6 (14%)	10 (24%)
Metabolic	157	66 (42%)	60 (38%)	25 (16%)	6 (1%)
Mutagenic	830	406 (49%)	284 (34%)	93 (19%)	47 (6%)
Immune response	44	8 (18%)	6 (14%)	14 (32%)	16 (36%)
Survival	43	7 (16%)	9 (21%)	16 (37%)	11 (26%)
Reproduction	55	17 (31%)	19 (35%)	13 (24%)	6 (11%)
Behavioral	81	10 (12%)	44 (54%)	18 (22%)	9 (11%)
Cancer	153	33 (22%)	45 (29%)	42 (27%)	33 (22%)
Disease	67	2 (3%)	12 (18%)	17 (25%)	36 (54%)
Damage	48	6 (12.5%)	21 (44%)	15 (31%)	6 (12.5%)
Developmental	37	8 (22%)	5 (13.5%)	11 (30%)	13 (35%)

[a] Number in parentheses is the percent of the total for that specific endpoint.

Table 12
Maximum stimulatory response in an inverted U-shaped curve by endpoint.

Endpoint type	Number of dose responses with ranges available	Maximum stimulatory response					
		⩾100 < 110% of control	⩾110 < 150% of control	⩾150 < 200% of control	⩾200 < 500% of control	⩾500 < 1000% of control	⩾1000% of control
Growth	3311	25 (0.8%)[a]	2175 (66%)	588 (18%)	423 (13%)	68 (2%)	32 (1%)
Metabolic	1839	5 (0.3%)	895 (49%)	395 (21%)	399 (22%)	79 (4%)	66 (4%)
Immune response	537	1 (0.2%)	272 (51%)	145 (27%)	100 (19%)	11 (2%)	8 (1%)
Survival	525	2 (0.4%)	397 (76%)	72 (14%)	45 (9%)	6 (1%)	3 (0.6%)
Reproduction	479	0 (0%)	292 (61%)	81 (17%)	76 (16%)	11 (2%)	19 (4%)
Neurological	265	0 (0%)	143 (54%)	67 (25%)	41 (15%)	9 (3%)	5 (2%)
Behavioral	185	0 (0%)	69 (37%)	41 (22%)	68 (37%)	5 (3%)	2 (1%)
Longevity	150	7 (5%)	82 (55%)	39 (26%)	22 (15%)	0 (0%)	0 (0%)
Mutagenic	62	1 (2%)	36 (58%)	10 (16%)	12 (19%)	1 (2%)	2 (4%)

[a] Number in parentheses is the percent of the total for that specific endpoint.

number of doses below the ZEP, etc.) have remained consistent. The data, in general, reveal that the literature basis is both quite extensive and broadly distributed across the range of biologically based sub-disciplines that utilize dose–response relationships. Previously it had been concluded that studies with more doses/concentrations resulted from the use of less expensive biological models such as plants. While this may have been true in earlier years when in vitro cell lines were hard to maintain and obtain, the shorter duration and ease of study designs have lead to more use of in vitro models, which in the database have more often been animal models. Although it is not clear whether the increase in in vitro animal models entered in the database is an artifact of subject interest, it is noted that there were more endpoints using in vitro animal models that demonstrate hormesis published in the last 10 years than any other time point.

The point system was developed along with the database in order to provide a framework to assess whether hormesis was a viable toxicological hypothesis. Although the point system has limitations, the database has provided the opportunity to assess the quantitative features of the dose–response curve. As was noted in the 2005 publication, the majority of the inverted U-shaped curves are 110–150% of the control with nearly 80% of the maximum responses less than 2-fold greater than the control. Therefore, the stimulatory responses, mainly in inverted U-shaped

Table 13
Distance from the maximum stimulatory response to the ZEP[a] by model.

Distance (-fold)	Number of dose–response relationships	Number in plants	In vitro studies in plants	In vivo studies in plants	Number in animals	In vitro studies in animals	In vivo studies in animals	Number in bacteria
⩾1 < 5	3331 (62%)[b]	1065 (69%)[c]	757	308	1905 (60%)	923	982	307 (58%)
⩾5 < 10	862 (16%)	257 (17%)	166	91	492 (16%)	191	301	99 (19%)
⩾10 < 100	837 (16%)	175 (11%)	98	77	543 (17%)	213	330	103 (19%)
⩾100	304 (6%)	57 (4%)	37	20	222 (12%)	65	157	21 (4%)
Total	5331 (100%)[d]	1554 (100%)	1058	496	3162 (100%)	1392	1770	530 (100%)

[a] ZEP = zero equivalent point (i.e., the highest dose showing a response equal to the control response).
[b] The number in parentheses is the % of total dose–response relationships.
[c] The number in parentheses is the % of total for that model.
[d] The value of 5331 differs from the total number of dose–response relationships (i.e., 8962) because calculation of the distance was not possible for all dose responses for various reasons (e.g., data presentation precludes exact determination of ZEP).

Table 14
Distance to the maximum stimulatory response to the ZEP by endpoint.

Endpoint type	Number of dose responses with distance available	Distance from maximum stimulatory response to the ZEP			
		⩾1 < 5	⩾5 < 10	⩾10 < 100	⩾100
Growth	1832	1167 (64%)[a]	308 (17%)	252 (14%)	105 (6%)
Metabolic	1128	621 (55%)	205 (18%)	232 (21%)	70 (6%)
Mutagenic	731	475 (65%)	107 (15%)	122 (17%)	27 (4%)
Immune response	323	157 (49%)	49 (15%)	64 (20%)	53 (16%)
Neurological	193	91 (47%)	37 (19%)	48 (25%)	17 (9%)
Survival	439	323 (74%)	61 (14%)	46 (10%)	9 (2%)
Reproduction	296	208 (70%)	43 (15%)	0 (0%)	13 (3%)
Behavioral	151	105 (70%)	25 (17%)	17 (11%)	4 (3%)
Cancer	78	60 (77%)	7 (9%)	9 (12%)	2 (3%)
Damage	36	28 (78%)	4 (11%)	3 (8%)	1 (3%)
Developmental	36	27 (75%)	5 (14%)	2 (6%)	2 (6%)
Longevity	82	64 (78%)	9 (11%)	8 (10%)	1 (1%)

[a] Number in parentheses is the percent of the total for that specific endpoint.

Table 15
Evidence of hormesis as used in the hormesis database[a].

Evidence of hormesis	Number of dose–response relationships	Percent of total dose–response relationships (8962)
High	859	10
Moderate–high	403	4
Moderate	907	10
Low–moderate	1616	18
Low	5137	57
No–low	40	0.4

[a] For a description of the quantitative methodology used to derive evidence of hormesis see Calabrese and Baldwin (1997a) and/or Table 1.

Table 16
The number of doses below the ZEP.

Number of doses	Number of dose responses (% of the 8962 dose responses)	Number of dose responses in plants	In vitro plant studies	In vivo plant studies	Number of dose responses in animals	In vitro animals studies	In vivo animal studies
1	1417 (16%)	472	142	330	804	362	442
2	1946 (22%)	697	198	499	1045	483	562
3	1975 (22%)	722	181	541	1056	513	543
4	1439 (16%)	491	132	359	805	433	372
5	979 (11%)	349	90	259	520	267	253
6+	1206 (13%)	435	130	305	638	452	186

curves, observed in the hormesis database still exhibit a modest magnitude and width in the majority of the cases. These features are biologically significant since they occur across biological models, endpoints, and chemical class/physical agent.

Although the majority of the dose responses in the database displayed low evidence of hormesis, the findings reflect both the strength of the data supporting hormesis as well as the evaluative criteria applied to the study design and response data and their evaluation (e.g., statistical analysis). The hormesis–frequency database (Calabrese and Baldwin, 2001b) provides an absolute judgment on whether the evaluative criteria of hormesis were satisfied or not. If such criteria were satisfied, then hormesis was judged as present. In contrast, the hormesis database, which is the subject of the present paper, makes no absolute judgment on the existence of

hormesis; instead, it applied different evaluative criteria, which result in dose responses being characterized according to the degree to which they are consistent with the hormetic–biphasic dose response. Given that these databases were constructed for different purposes and used different evaluative criteria, it is important to note that when all 245 dose–responses that satisfied the evaluative criteria (i.e., hormesis) in the hormesis–frequency database were assessed using the scoring system employed on the dose responses in the hormesis database, the distribution of the ranked scores were similar (Calabrese and Blain, 2005). This is significant since it was our strong general impression before conducting this inter database comparison that the entry criteria of the hormesis–frequency database (Calabrese and Baldwin, 2001b) was considerably more stringent than the hormesis database, but this is not the case.

The overall findings indicate that the quantitative features of the hormetic dose response are consistent across biological model and endpoint. These observations suggest that this feature has been highly conserved within an evolutionary context. It also suggests that the hormetic dose response may provide a quantitative estimate of biological plasticity that is broadly generalizable (Calabrese and Mattson, 2011; Calabrese, 2010).

Conflict of interest statement

The authors declare that there are no conflicts of interest. This manuscript has not been published previously and is not under consideration for publication elsewhere.

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Hormesis and medicine

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Evidence is presented which supports the conclusion that the hormetic dose–response model is the most common and fundamental in the biological and biomedical sciences, being highly generalizable across biological model, endpoint measured and chemical class and physical agent. The paper provides a broad spectrum of applications of the hormesis concept for clinical medicine including anxiety, seizure, memory, stroke, cancer chemotherapy, dermatological processes such as hair growth, osteoporosis, ocular diseases, including retinal detachment, statin effects on cardiovascular function and tumour development, benign prostate enlargement, male sexual behaviours/dysfunctions, and prion diseases.

Introduction

The following paper will make the case that the hormesis concept is the most fundamental dose–response in the biomedical and toxicological sciences. Over the past decade there has been a remarkable surgence of interest in hormesis as a result of more significance being given to low dose effects and the use of more powerful study designs which have permitted the detection of the hormetic biphasic dose response in the low dose zone. This paper will establish the occurrence of hormesis within the biomedical literature, its quantitative features, mechanistic foundations, and applications to the field of clinical pharmacology. The hormetic dose–response challenges long-standing beliefs about the nature of the dose–response in a low dose zone and has the potential to affect significantly the design of pre-clinical studies and clinical trials as well as strategies for optimal patient dosing in the treatment of numerous diseases. A detailed historical assessment of how and why the hormetic dose response became marginalized in the biomedical literature was published by Calabrese in 2005 [1].

The hormetic dose–response relationship

We created an hormesis database for articles published in the peer-reviewed literature using *a priori* evaluative criteria in order to assess more systematically and objectively this concept. The criteria take into account the strength of the study design features, magnitude of the low dose stimulation, statistical significance and reproducibility of the findings. To date there are approximately 8000 dose–responses within this relational retrieval database. A detailed description of the database was published in 2005 [2].

The hormetic dose–response may be reliably described as a being a stimulation in the low dose zone, followed by an inhibitory response at higher doses. The magnitude of the stimulatory response at maximum is typically modest, being only about 30–60% above that of the control response (Figure 1). The strong majority of stimulatory responses are less than twice the control value. This is the most distinguishing characteristic of the hormetic dose–response, being its most consistent and reliable feature.

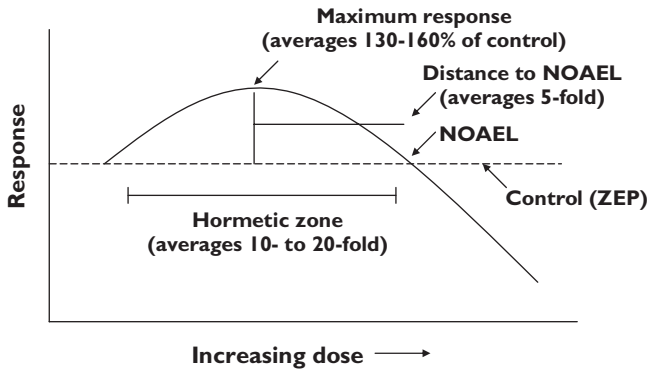

Figure 1

Dose–response curve showing the quantitative features of hormesis

The width of the stimulatory response is typically within 100-fold of the zero equivalent point, that is, the dose where the response changes from stimulation to inhibition, (i.e. the threshold value). In a small proportion (i.e. <2%) of the hormetic dose responses analyzed to date, a very broad stimulatory dose–response range has been noted, exceeding 1000-fold. The implications of having a wide stimulatory zone may be clinically significant. For example, the stimulatory zone defines the therapeutic window. It may also define an adverse effect window, as in the case when low doses of anti-tumour drugs stimulate tumour growth [3]. It is also important to recognize that the hormetic stimulatory zone is graphically contiguous with the pharmacologic/toxicologic threshold. This indicates that there is the distinct possibility of a desired therapeutic dose being a toxic dose to some individuals due to extensive interindividual variation.

The hormetic dose–response can occur (i) as a direct stimulatory response, (ii) after an initial disruption in homeostasis followed by the modest overcompensation response, or (iii) as a response to an 'adapting' or 'preconditioning' dose that is followed by a more massive challenging dose. The relationship of hormesis to the adaptive response was explored by Davies *et al.* [4] who defined the optimal condition for a transient hydrogen peroxide adaptation as measured by cell viability in the yeast model *S. carerviase*. In a critical first step, the authors determined the effect of hydrogen peroxide employing up to nine concentrations and a variety of cell densities. Of particular note was the observation of an hormetic-like biphasic dose-response in which low hydrogen peroxide concentrations (≤0.4 mM) enhanced cell colony growth by approximately 30%. The hydrogen peroxide-induced toxicity started to occur between 0.5 and 0.8 mM. Based on these findings an adapting or preconditioning dose was selected to be one in the low dose stimulatory/hormetic zone. The yeast cell that received the adapting dose in the hormetic zone followed by the challenging (i.e. cell killing) dose not only showed the adaptive response but also displayed a percent viability that exceeded the original control value by approximately 20–50%.

Since the quantitative features of hormetic dose–responses are similar regardless of the biological model, gender, endpoint measured and inducing agent as well as whether it occurs via direct or overcompensation stimulation, it suggests that such features maybe a quantitative index of the plasticity of biological systems. If this is the case, it would have important implications for the field of clinical pharmacology by placing biological constraints on the magnitude of the increase in performance one could expect from drug treatments.

Since the hormetic effect is one that is highly generalizable across biological models, it suggests that this response strategy has been highly selected for, indicating that the hormetic dose–response is adaptive in nature. This is particularly seen in the context of adaptive/preconditioning responses where a prior exposure to a low dose of a toxic agent or stressful condition up-regulates adaptive mechanisms that protect against subsequent exposures to similar toxic agents or stressor conditions. The duration over which such protection occurs will vary according to the biological model and endpoint. However, it usually does not extend beyond about a 10–14 day period [5].

Since hormetic dose–responses are highly generalizable, occurring in essentially all species of plants, microbes, invertebrates and vertebrates, in all organ systems, and for a large number of endpoints, there is a generalized mechanistic strategy but no single mechanism. The 1977 paper by Szabadi [6] suggests one such general strategy which may account for numerous cases of hormetic-like biphasic dose–response relationships. In this case, a single agonist may bind to two receptor subtypes, with one activating a stimulatory pathway while the other activates an inhibitory one. The receptor subtype with the greatest agonist affinity would typically have the fewer receptors (i.e. lower capacity) and its pathway activation effects dominate at lower doses. Conversely, the second receptor subtype would have lower agonist affinity, greater capacity (i.e. more receptors) and become dominant at higher concentrations. This generalized scheme of Szabadi [6] has been supported [7–9] over the past three decades and is able to account for numerous cases of direct stimulation hormetic dose–response relationships. It may also be directly applicable to situations in environmental toxicology in which the toxin induces dose-dependent changes in the concentrations of various endogenous agonists, a situation which is known to commonly occur. In such cases one would readily expect the occurrence of an hormetic-like biphasic dose–response relationship.

Hormesis and interindividual variation

A principal concern in assessing the effects of drugs on humans is that of inter-individual variation. Numerous

factors are known that contribute to such variation, including age, familial background, gender, nutritional status, the presence of pre-existing disease, amongst other factors. Using the hormesis database we subsequently identified a substantial number of experimental settings in which hormesis had been studied in individuals or closely related strains of organisms which differed in susceptibility to toxic agents. In these evaluations we compared responses where the range in susceptibility varied from less than 10 fold to well in excess of 100 fold [10]. Of particular note was that the hormetic response was generally independent of susceptibility, with hormetic responses occurring in subjects ranging from high to low susceptibility. Likewise, the quantitative features of the hormetic dose–response are independent of susceptibility. In about 20% of the cases, it appeared that the lack of an hormetic response in a susceptibile strain or subgroup was related to its increased risk. These observations have important implications in the development of treatment strategies for patients.

Drug interactions

Hormesis contributes a new dimension to the concept of chemical interactions. Hormetic dose–responses describe that portion of the dose–response that relates to performance, that is, the portion of the dose–response immediately below the threshold. This is in contrast to the portion of the dose–response above the threshold, the location where most examples are drawn for illustration purposes to demonstrate the potential magnitude of toxic interactive effects [11]. The magnitude of the hormetic dose–response interaction will also be constrained by the bounds of biological plasticity. The magnitude of the hormetic interaction will also be only 30–60% greater than the control value. The interaction is principally seen within the context of the reduction in dose that is necessary to achieve a strong interaction response. This was described in considerable detail in several papers by Flood *et al*. [12, 13] dealing with memory. These authors recognized that one of the important implications of these findings was that this would reduce the likelihood of adverse effects because of the very low doses of drugs needed to achieve the therapeutic effect.

Medical implications of hormesis

Anxiolytic drugs

The animal model testing of anti-anxiety drugs became extensive in the 1980s [14–18] with progressive methodological advances continuing to the present. In the screening of potential drugs the goal has been to reduce anxiety in the mouse or rat model in specific experimental settings. While there are many ways in which this problem has been studied, the basic strategy is to assess whether the drug treatment can result in the mouse and/or rat performing specific behaviours that they would normally resist or not be inclined to do. In rodent experiments it can be trying to get the animals to spend more time in lighted as compared with dark areas. The possible anxiolytic agent may be tested in a maze like apparatus in which there is choice between entering and exploring a dark or a lighted alley. If the drug increases the time spent in the lighted alley or the number of entries into the lighted alley per unit time then the drug would be judged as anxiolytic. There are more than a dozen commonly employed anxiolytic tests (e.g. elevated plus maze test, hole board test, light-dark test, social interaction test, four plates test, open-field test, staircase test, conflict test, forced swimming test, tail suspension test) for screening drugs using animal models that address a wide range of behaviours that would be indicative of anxiety, relating to dark and lightness, social interactions, social conflicts, and a variety of other aversion behaviours, all directed toward slightly different manifestations of anxiety. While the basic premise is to determine if the drug can make the animal do that which is uncomfortable, each test is unique, providing an evaluation of somewhat different behaviors.

The most common dose–response relationship seen for the broad spectrum of anxiolytic drug screening tests is the hormetic model (see [11, 211] for a review), regardless of the chemical class, strain of the animal model or gender tested [19]. Therefore, anti-anxiety drugs regardless of their class will generally not increase the so-called 'anti-anxiety' response (i.e. decreased anxiety) by more than about 30–60% at the optimal dose. There can be large differences in the potency of anxiolytic drugs, sometimes differing over several orders of magnitude of dose. However, the general quantitative features of the hormetic dose–response are the same regardless of the potency of the agent (Figure 2).

Anxiolytic drugs have been shown to act through a wide range of receptors (e.g. 5-HT, dopamine, adenosine, GABA, NMDA) that mediate the response, thereby reducing anxiety via a variety of proximate mechanisms. Despite the fact that these drugs reduce anxiety by different proximate mechanisms they still show the same hormetic dose–response relationship, with the same quantitative features.

The hormetic dose response therefore is an important feature by which anxiolytic drugs act, being the basis for why the drug was selected for clinical evaluation, as well as being independent of animal model, gender, potency, and mechanism of action. This is a powerful set of parameters that converge around the hormetic dose–response relationship making it a central concept in the discovery and assessment of anxiolytic drugs.

Anti-seizure drugs

Another example where hormetic biphasic dose–response relationships have played an important role in drug discovery concerns anti-seizure medications. As in the case of anxiolytic agents, seizure drugs also undergo a screening

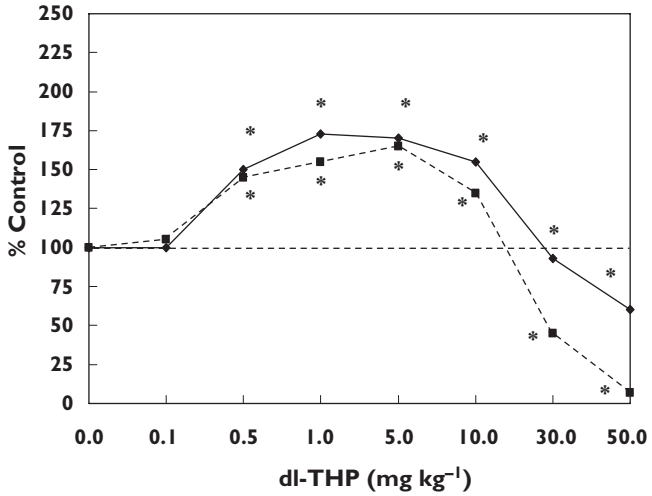

Figure 2

Anxiolytic effect of dl-THP, a naturally occurring alkaloid, on ICR mice of both sexes in the elevated plus-maze test. *Significantly different from controls at $P \leq 0.05$ [207]. % Open arm entries, (—◆—); % Time in open arm (- -■- -)

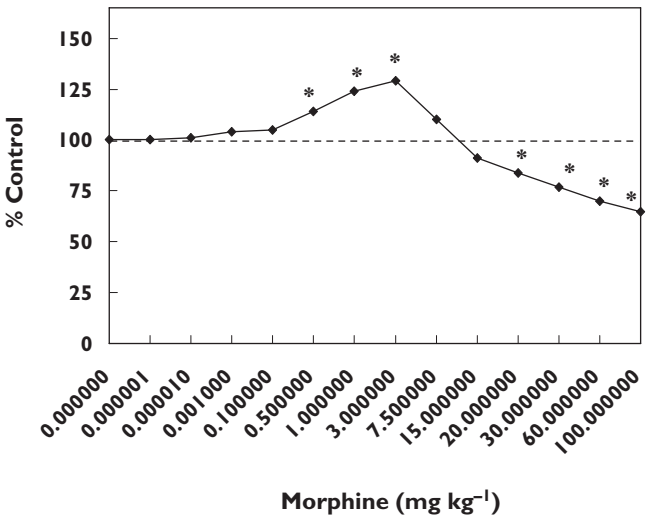

Figure 3

Effect of different doses of morphine on PTZ-induced seizure threshold. *Significantly different from controls at $P \leq 0.05$ [208]

process to eliminate the poorer performing agents and to identify those with clinical potential. One way that this is achieved is to induce seizures in animal models with standard seizure-inducing agents [e.g. pilocarpine, flurothyl-(hexafluorodiethyl) ether, kainic acid, and pentylenetetrazol (PTZ)]. When this is done the researcher determines the dose required to induce a certain frequency of seizure events within a specific period of time. Drugs thought to have good potential as anti-seizure agents would be those that demonstrate the capacity to increase significantly the threshold dose of the model drug that causes seizures. That is, if the threshold dose for inducing seizures in the animal model is 100 mg kg^{-1}, then a potential anti-seizure drug would make it more difficult for that model drug to induce the seizure response, that is, requiring even higher doses (i.e. increasing the response threshold).

The hormesis concept directly relates to how anti-seizure agents are detected in the above screening process. In the course of evaluating anti-seizure agents investigators typically assess the agent across a broad range of doses. In such evaluations anti-seizure agents at low doses increase the seizure threshold of the model seizure-inducing drug while at higher doses the anti-seizure drug typically enhances the occurrence of seizures by lowering the threshold of response. In Figure 3 morphine induced an hormetic-like biphasic dose–response on the PTZ-induced seizure threshold. Note that the threshold dose for the PTZ induced seizures increased by approximately 25%. In essence, these anti-seizure drugs follow the pattern of the hormetic biphasic dose–response relationship (see [11, 212] for a review). The extent to which the threshold increases also conforms to the quantitative features of the hormetic dose–response, usually increasing at maximum only within the 30–60% zone above the control.

Learning and memory enhancement

The first major efforts to explore whether drugs could enhance learning in animal models were undertaken at the University of California at Berkeley in the Department of Psychology during the 1960s. While these efforts extended earlier preliminary investigations at the University of Chicago and elsewhere, the Berkeley group created a new research direction that lead to the development of valuable drugs in the treatment of cognitive disabilities as seen with Alzheimer's (AD) and related diseases of ageing. In fact, the initial breakthrough was undertaken by then two graduate students (James McGaugh and Lewis Petrinovitch), who hypothesized that memory was related to the concentrations of acetylcholine released by the neurons. With this guiding framework these students tried to determine why some mice were bright (i.e. smart) and others were dull (i.e. not so smart). To test this hypothesis they administered a drug over a broad dose range to the bright and dull mice that would prevent the normal breakdown (i.e. hydrolysis) of the acetylcholine. The agent used to slow down the normal breakdown of acetylcholine was physostigmine, a natural constituent of the Calabar bean, with a long history of use in medicine, especially known to cause contraction of the pupils. The treatment was expected to make the dull mice brighter and the bright mice even brighter, but only up to a point, that is, when the dose exceeded a hypothetical optimal zone, triggering a decline in performance. Both the dulls and brights exhibited the characteristic U-shaped dose–response relationship (Figure 4), thereby confirming the study hypothesis. The manuscript based on these findings was rejected with the

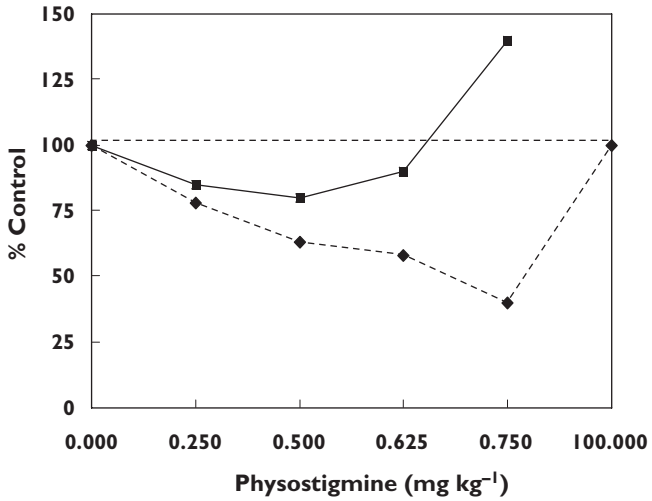

Figure 4

Median trials to criterion for maze bright and maze dull groups [209]. Brights, (- -■- -); Dulls, (—◆—)

stated reason that it was well known that chemical intervention could only decrease learning and memory, not enhance it. The two students persevered, publishing their paper several years later, opening up a new era in the psychology and pharmacology of learning and memory research.

The work of McGaugh and Petrinovitch propelled an intellectual revolution in the neurobehavioural sciences for understanding of memory and the exploration of therapies for those with cognitive dysfunctions. The idea that one could improve learning by inhibiting the breakdown of acetylcholine was important as it established an intellectual platform for subsequent research. This was also occurring at a time when organophosphate and carbamate insecticides were being developed as agents to kill insects, with both groups of chemicals inhibiting the enzyme that hydrolyzed acetylcholine. In fact, in some of the subsequent research it was shown that administration of some of these agents appeared to increase learning and memory in the rodent models. While it has never been seriously proposed that such pesticides could be used to boost memory in people, much work was undertaken with physostigmine in clinical settings [20–50]. During this period of little more than 15 years there were about 20 AD clinical studies published involving several hundred individuals. The findings consistently noted a modest (i.e. 10–30% range, generally) improvement in various types of memory. Despite the capacity of physostigmine to enhance memory performance, it was generally accepted that the modest increases did not offer enough change to provide dramatic or often even practical improvements in patients' lives, although there were some reports where greater degree of independent living was seen. This is especially the case given the pharmacokinetic features of this drug, with its very short half-life, requiring up to five doses per day for most subjects.

While these agents pointed the way for future research, there was the need for second and third generation agents, all with markedly improved pharmacokinetics profoundly lowering risks of side effects. Amongst those drugs extensively evaluated in this regard have been tacrine [13], heptylphosphostigmine [51], huperizine A [52, 53], arecoline [12] and gastigmine [54], all being based on an anti-ACHE concept following the premise that McGraugh and Petrinovich employed with physostigmine. The dose–response relationship for each drug was repeatedly demonstrated to be a U-shaped in multiple animal models much like that seen in Figure 4. Such findings lead to the general conclusion that the therapeutic window for such drugs in the treatment of AD patients followed the U-shaped dose–response that was causally linked to the percent of ACHE inhibition. However, more recent investigations have revealed that the neuroprotection is likely to involve multiple mechanisms, either in addition to ACHE inhibition or independent of it. Regardless of the underlying mechanistic explanation and how it may differ amongst protective agents, the quantitative features of the dose–response remain similar (see [213] for a review).

Four (i.e. Aricept, Cognex, Exelon and Razadyne) of the five drugs that have been approved for the treatment of AD by the US FDA are based on the inhibition of ACHE and demonstrate the hormetic dose–response, with the low dose stimulation being key to the increased memory improvement. The fifth approved drug, memantine, an NMDA antagonist, also acts via an hormetic-like inverted U-shaped dose response relationship [55]. Yet it is also important to recognize that the constraints of the hormetic dose–response indicates that the expected increase in performance is likely to be modest at best, observations confirmed with substantial clinical experience.

Anti-tumour drugs

A major goal of anti-tumour drug screening is to find agents that are effective in killing a broad range of tumour cell types. Those who focus on such concentration responses are typically estimating responses at the high end of the concentration–response relationship, then trying to determine the mechanism by which the killing occurred. While acknowledging the critical importance of this perspective, our interests with respect to hormesis are different, that is, focusing on the low concentration end of this relationship, that is, the zone starting immediately below the toxic threshold.

In a recent assessment Calabrese [3] provided substantial documentation that low doses of anti-tumour agents commonly enhanced the proliferation of the human tumour cells, in a manner that was fully consistent with the hormetic dose–response relationship. These hormetic dose–responses were occurring for most types of tumour cells, independent of organ. That is, Calabrese [3] reported

hormetic dose–responses in 138 *in vitro* cell lines, and 32 primary human types induced by over 120 agents with nearly 50 being endogenous agonists while others involved the effects of various drugs, phytochemicals, and environmental contaminants.

While many of the researchers did not focus on the low dose stimulatory responses provided in their tables and figures, choosing to address only the higher concentration effects, others not only noted these findings but attempted to account for them in follow up mechanistic studies. In some instances there was a stronger interest in the clinical implications of such findings, suggesting that the low dose stimulation of human tumour cell proliferation could be potentially problematic for patients who have been treated with chemotherapeutic agents. A case in which the implications of low-dose stimulation of tumour cell growth has been extensively assessed is that of dexamethasone and neuroepithelial tumours. Dexamethasone has long been employed to prevent swelling in the brain after removal of a brain tumour [56, 57]. While this treatment is important in the management of such patients, a number of *in vitro* experimental studies using dexamethasone have consistently demonstrated that specific types of neuroepithelial tumours proliferate with dose–response features consistent with the 30–60% maximum stimulatory response. This low-dose enhanced response required the presence of a glucocorticoid receptor. Lacking such a receptor prevents the dexamethasone induced low-dose stimulatory response [58, 59].

Upon the publication of a paper by Kawamura *et al*. [60] assessing this type of biphasic response, the journal editor invited expert commentaries from other leading neuroepithelial surgical researchers on the implications of treating surgical brain tumour patients with dexamethasone [61–65]. Despite concerns raised by the experimental data and the expert commentaries, it was not clear what the tumour growth implications would be following surgery. No attempts have been published providing quantitative modeling of various possible clinical scenarios.

If tumour cells remain after treatment these data suggest the possibility that some chemotherapeutic agents could promote tumour growth once the concentration of agent entered into the low dose stimulatory zone. This would be a particular concern for agents (e.g. suramin) (Figure 5) with a very long half-life [66], while being less of a problem for agents with very short half-lives. As with other examples of hormetic dose–responses, the magnitude of the enhanced proliferation was typically in the 30–60% range at maximum. What implications this might have for tumour growth over a longer time period has not been assessed.

The hormetic concentration–response observations in the human tumour cell lines in the peer-reviewed literature is mirrored in the US NCI anti-tumour agent databases. There are two databases that we have studied in some depth, the human tumour cell line database which is

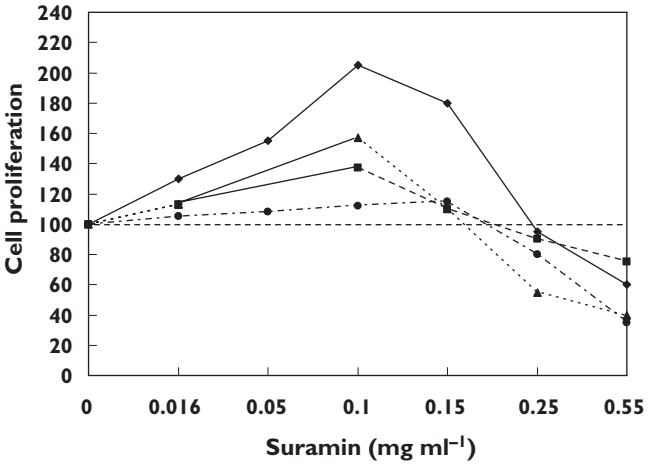

Figure 5

Effect of suramin on human breast cancer cells *in vitro* [66]. ZR/HERc Cells, (—◆—); ZR75.1 Cells, (- -■- -); T-47D Cells, (- -▲- -); MDA-MB-231 Cells, (- -●- -)

comprised of at least 60 different tumour cell lines representing tumour types from many areas of general clinical significance. The second database is comprised of 13 strains of yeast that have genetic defects relating to DNA repair and cell cycle control.

We have published a detailed analysis of the yeast data set that contains over 56 000 concentration–response studies [67]. This evaluation tested whether the hormetic or the threshold dose–response model best predicted the responses of the chemicals in the low dose zone. These analyses revealed that the threshold dose–response model poorly predicted responses at low concentrations. On the other hand, the hormetic dose response model performed very well, accurately predicting low concentration responses. The findings suggested that clinical oncologists need to become more cognizant of the possibility that treatments designed to kill tumour cells or suppress their proliferation in patients may have the capacity to enhance tumour growths when the drug eventually reaches a low (i.e. stimulatory) concentration in the body, in the days after the chemotherapy is administered.

Stroke and traumatic brain injuries

Given their potentially debilitating consequences strokes are a major public health concern. Considerable research has been devoted to enhance the understanding of the causes of strokes within the population in order to reduce their frequency and severity. Familial history, high blood pressure that is ineffectively treated, smoking, and excessive stress are some of the known risk factors affecting stroke.

These concerns have led numerous researchers to assess possible treatments of stroke with a wide range of

animal models. Such studies, which typically involve the induction of one of a variety of specific types of stroke that occur with substantial frequency within humans, have often yielded findings of encouraging neuroprotection, only to fail once the agents were subjected to clinical trials, yielding a very low successful transition to the marketplace. There has been considerable discussion about why there has been such a disconnect between successful preclinical studies and failures of a large number of clinical trials. Amongst the many reasons for such failures in the stroke area include concerns over the validity of animal models to predict human responses in a sufficiently reliable manner, inadequately designed clinical trials, especially factors such as the entry criteria for patients may lack the necessary precision for proper comparisons, the practical problems of long delays between the onset of stroke symptoms and therapy, amongst other factors. A factor that has often gone unnoticed is the dose–response relationship of the therapeutic agent. In fact, as in many other medical conditions, the shape of the dose–response of the treatment can often take that of a U-shape, that is, showing characteristics of an hormetic dose–response. However, this type of dose–response is not usually seriously considered as far as the clinical trial is concerned. Since the biomedical and clinical domains have been so long dominated by the assumed sigmoidal nature of the dose–response, investigators have often assumed that the response for a drug may be enhanced by pushing the dose ever higher, up to a point when the response tends to flatten out [68].

Despite the fact that U-shaped dose–responses have been reported in stroke related preclinical investigations this information had never been integratively summarized, until recently [69]. U-shaped dose–responses have been reported for nearly thirty different agents in the treatment of stroke related brain damage [70, 71] and in studies directed to blunt traumatic brain injury [72, 73], both of which share similar common damage-inducing mechanisms. Different drugs that have been successfully employed in these studies are usually selected based upon a certain hypothesized mechanism by which stroke or traumatic damage is mediated. If the drug can turn off the damage mechanism switch or turn on a repair mechanism switch that could alleviate the damage then protection would be observed. Since there are many stages in the process leading to final tissue damage, intervention at separate and key points in this scheme would prevent significant damage from occurring. That is in fact what has occurred in these studies. Many experimental plans have worked well, leading to damage reduction, and essentially all displayed the same type of hormetic dose response regardless of where in the disease process the intervention occurred.

Since the U-shaped dose–response is commonly observed in these chemoprevention studies such as discussed above, it needs to be anticipated better and explored in order to enhance the success of getting new and effective therapeutic agents through the testing and evaluation process.

Benign prostate hyperplasia/cardiac glycosides

The use of digitalis in the treatment of congestive heart disease has been one of considerable historical interest with its first reporting in 1775, being credited to the British physician William Withering [74–76]. Over time it was determined that cardiac glycosides, such as digitalis, most likely acted by blocking the sodium pump (i.e. Na^+/K^+-ATPase) at dosages that are achieved *in vitro* or intravenously. Biomedical understandings of the dose–response for these agents on the sodium pump and their broader range of effects started to become considerably more complex in the mid 1970s. At this time investigators reported that cardiac glycosides affected the sodium pump biphasically, that is, stimulating activity at low concentrations while being inhibitory at higher concentrations [77]. These initial findings revealed the existence of high and low affinity binding sites, with the high affinity sites having only 3% of the capacity of the low affinity one. The binding of agents, such as ouabain, to both high and low-affinity sites has been linked to the sodium pump and/or functionally distinct Na^+/K^+ pumps and proposed to account for its biphasic dose–response activities, findings that have been subsequently reinforced [78, 79].

Of particular interest is that cardiac glycosides were also reported to enhance proliferation of a broad range of cell types in a biphasic fashion, starting in the mid 1970s for immune cells [80, 81]. The range of biphasically affected cell types includes smooth muscles from the canine saphegeous vein [82], smooth muscle from the human umbilical vein [83], smooth muscle from the adult prostate of subjects with benign prostate hyperplasia (BPH) [84], smooth muscle from a rat cell line (A7r5) which was selected for study due to a specific protein component of the sodium pump [83], mature human red blood cells which were selected for study due to membrane properties [85], rat renal epithelial cells [86] as well as HeLa cells which were selected for study due to their unique sensitivity to digitalis induced toxicity [87]. These diverse experimental studies have centered in large part on the development of improved understandings of the sodium pump and its capacity to function in ways that may not involve the well established ionic shift, suggesting a multiplicity of functions for the sodium pump, including diverse message signalling with broadly expanding clinical implications.

The quantitative features of the biphasic dose–response of cardiac glycosides indicate that they are fully consistent with the hormetic dose–response model. Figure 6 provides a description of the dose–response features of various cardiac glycosides in a range of biological

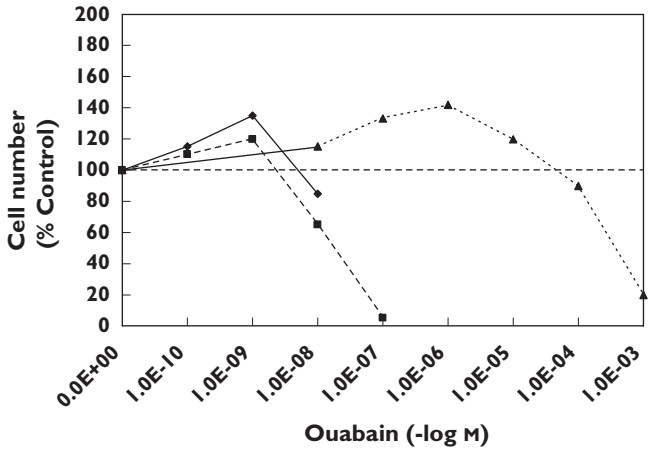

Figure 6

Comparison of the effect of ouabain on hormetic dose–responses in three biological models [83]. Canine (CVSMC), (—◆—); Human (HUVSMC), (- -■- -); Rat (A7r5), (· · ▲ · ·)

models adjusted to the same scale for comparison purposes [83]. In each case the magnitude of the low dose stimulation is modest, with the maximum stimulation typically being in the 30–60% range above the controls. The width of the stimulatory response was also generally consistent across specific agents and biological models and usually less than 100-fold, again consistent with the hormetic dose–response.

Several of the above studies have obvious implications for clinical pharmacology. In 2001 Chueh *et al*. [84] reported that concentrations of ouabain in the therapeutic zone for cardiac glycosides enhanced cell proliferation of smooth muscle from the prostate gland of subjects with BPH. The magnitude of the increase was modest, approaching only about 20% above control values. However, these authors indicated that even such modest increases could have important clinical implications since such smooth muscle comprises about 40% of the area density of BPH tissues. Several reports have indicated that a small increase in prostate size significantly affects clinical symptoms in patients with BPH. In a similar fashion, a modest reduction in prostate volume of only about 30% has been repeatedly demonstrated to improve markedly clinical symptoms [88, 89]. Thus, the hormetic biphasic dose–response that was reported in these *in vitro* investigations is suggestive of possible clinical implications.

Studies with HeLa cells by Ramirez-Ortega *et al*. [87] revealed that four digitalis compounds biphasically affected cell proliferation, again in a manner consistent with the hormetic dose–response. In this case, the concentration ranges over which the stimulatory response range occurred were very broad and remain to be clarified. That is, the stimulatory response range was greater than 1000-fold, and could be within a concentration range approaching that of endogenous cardiac glycosides. While the biphasic dose–response was reported for four digitalis-like compounds it was not seen for digitalis when tested over similar concentrations due to its greater toxicity. Of particular mechanistic importance was the fact that the low dose stimulation occurred even in the presence of ethyacynic acid, a nonsteroidal inhibitor of Na^+/K^+-ATPase, thereby suggesting that the stimulation is independent of the sodium pump. Finally, even though the authors did not address the clinical implications of these findings it would appear that a broad range of digitalis compounds have the capacity to enhance cellular proliferation in numerous cell types including human tumour cell lines over a concentration range that encompasses the therapeutic zone as well as for possible normal background states.

Statins: CVD and cancer

Statins have become important drugs in the prevention of cardiovascular disease. They are also considered potentially significant in the treatment of numerous types of solid tumours and for other diseases (e.g. diabetic retinopathy and macular degeneration) that depend on capillary development. Some investigators suggest potential clinical application for statins in the treatment of AD, osteoporosis [90] and other diseases. A converging conceptual framework that is emerging on the effects of statins in this broad range of tissues and endpoints is that they often display an hormetic-like biphasic dose–response relationship, a response that was unexpected, initially overlooked, but now a factor for optimizing patient treatment strategies.

The idea that statins may act via biphasic dose–responses emerged with the publication in 2002 by Weis *et al*. [91]. Using primary human adult dermal microvascular endothelial cells (HMVECs) and an immortalized human dermal microvascular endothelial cell line (HMEC-1), these authors demonstrated that cerivastatin (CEV) biphasically affected endothelial cell (EC) proliferation, migration, and apoptosis (Figure 7). They also demonstrated that CEV/ATR (atorvastatin) significantly reduced lung tumour growth at high doses, a response probably related to a marked decrease in tumor capillary development. The low dose stimulatory response was consistent with their hypothesis that HMG-CoA reductase inhibition would enhance endothelial processes involved in angiogenesis and could reverse impairments associated with cardiovascular disease.

The biphasic dose–response was independently confirmed about 2 months later by Urbich *et al*. [92] who had submitted their paper for publication consideration more than 2 months prior to the Weis *et al*. group. However, their paper was not accepted for publication until about 3 months after the Weis *et al*. paper. Using human umbilical vein endothelial cells (HUVECs) these authors also reported that ATR biphasically affected endothelial cell migration and tube formation. Similar findings were subsequently

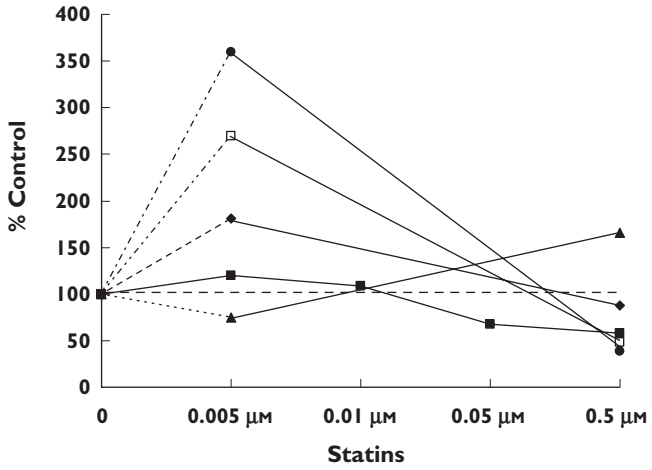

Figure 7

Effects of statins on human endothelial cells [91]. CEV EC Proliferation, (—■—); ATV EC Migration, (- -◆- -); CEV Apoptosis, (· ·▲· ·); CEV Tube Length, (- -● -); ATV Tube Length, (- -⊡· ·)

reported by Katsumoto *et al.* [93] with HMVECs for multiple endpoints including cell migration, chemotaxis, cell proliferation, trypan blue exclusion and others. Cooke [94], a co-author of the Weis *et al.* [91] paper, suggested that these findings indicate that statins may also enhance cardiovascular functioning by promoting angiogenesis in ischaemic limbs, that is, facilitating the remodeling of vessels toward a more normal and healthier condition. However, at higher doses these same agents display anti-angiogenic effects that are mediated by blocking L-mevolonate metabolism, reducing the synthesis of G-protein subunits. This process leads to an increase in apoptosis. The data of Weis *et al.* [91] and the other supportive findings noted above were striking, suggesting that statins could have 'puzzling' effects on angiogenesis – that is, proangiogenic effects at low therapeutic doses but angiostatic effects at higher doses [95].

Statin concentrations can broadly vary in the serum of patients, from a low of about 0.002 to a high of approximately 0.1 μmol l^{-1}. The low concentration stimulation for endothelial cell parameters ranges from less than 0.001 to about 0.01 μmol l^{-1}. Above 0.1 μmol l^{-1} high concentration effects start to become evident. The low concentration stimulatory effects (i.e. pro-migration, pro-angiogenic) of ATR were associated with phosphatidylinositol 3-kinase-AKT-pathway activation with phosphorylation of AKT and endothelial NO synthase (eNOS) at Ser 1177. Thus, the issue of whether statin concentrations are pro- or anti-angiogenic began to be raised and debated. However, from the available data it appears that the therapeutic concentration for lowering cholesterol concentrations spans the pro- and anti-angiogenic concentration continuum.

Proceeding along a separate intellectual track researchers interested in inhibitors of angiogenesis had not focused on the statins, which were principally seen as inhibitors of cholesterol synthesis. These researchers, following the lead of Folkman [96], identified a series of possible agents starting with interferon α (1980), plasminogen factor (1982) and some related hydrolyzed protein fragments, collagen derived endostatin and other endogenous agents, acting via a range of possible mechanisms, that inhibit angiogenesis. In the case of endothelial migration and other endpoints related to capillary development, such agents often displayed biphasic dose–response relationships. Furthermore, when tested in cancer bioassays, these anti-angiogenic agents generally displayed a U-shaped dose–response relationship [97]. In fact, even when tests failed to produce the expected U-shaped dose–response in cancer bioassays, as in the case of Eisterer *et al.* [98], it was most likely due to the treatment being at the high end of the U-shaped dose–response.

The mechanisms by which these U-shaped dose–responses occur has been generally viewed as 'unclear' [99], 'uncertain' [100], or 'still to be elucidated' [101]. Various possible mechanistic explanations have been suggested including the induction of receptor dimerization that could lead to biphasic effects or the induction of multiple signaling pathways that display an integrated U-shaped dose–response.

A complementary and/or alternative perspective on anti-angiogenic agents has been developed by Jain and colleagues [102] based on the proposal that normalization of tumour vasculature can mediate anti-angiogenic cancer therapy. This group has shown that tumour vasculature is very different from normal tissue. That is, tumorous vascular tissue can be very irregular with respect to bifurcation and the sizing of the bifurcating vessels. Vessel walls can be very tight or very leaky or intermediate. Pressure gradients within the vessels are also highly variable. The blood flow can also be very irregular with flow going in back and forth directions, or simply being stagnant. The oxygen concentrations are also usually very low, that is, hypoxic, with high acidity being common. This condition reduces the efficiency of immune cells to attack the tumour while enhancing the capacity of tumour tissue to metastasize.

Jain and colleagues [102] reduced the cancer angiogenesis capacity by using an antibody against VEGF, a promotional signal molecule abundant in most solid tumours. This treatment caused some of the aberrant tumor vessels to be 'punned' away. This treatment also affected a remodeling of the remaining tumour vessels toward a more normal state, becoming less leaky, dilated and tortuous. There were also higher concentrations of oxygen in the tissue and enhanced penetration of chemotherapeutic agents.

The idea of remodeling tumour blood vessels toward a more normal state seems contrary to the goal of Folkman [96, 97] to kill the tumours by preventing capillary development. The anti-angiogenic drug Avastin increased survival only in combination with standard chemotherapy, possibly because it pruned some of the aberrant blood vessels, remodeling the remaining ones, making them

more susceptible to chemotherapy. Fukumura & Jain [102] believe that it is important to identify the time period when the vessels become remodeled in order to determine the optimum period of chemotherapy. The possibility exists that anti-angiogenic agent-induced pruning and remodeling may represent a biphasic dose–response, related to the findings of Weis *et al*. [91] and Urbich *et al*. [92] and later authors.

In summary, anti-angiogenic agents typically follow a U-shaped dose–response that displays the quantitative features of the hormetic dose–response. The U-shaped dose–response has become a common theme within the past 5 years of statin research, and is consistent with other effects of anti-angiogenic agents such as their effects on bone development [90]. While the mechanisms by which such hormetic effects occur for each endpoint are likely to differ, this should be seen as a basic regulatory strategy by which the biological system balances pro and anti-angiogenic signals within a framework of the constraints imposed by system-related biological plasticity.

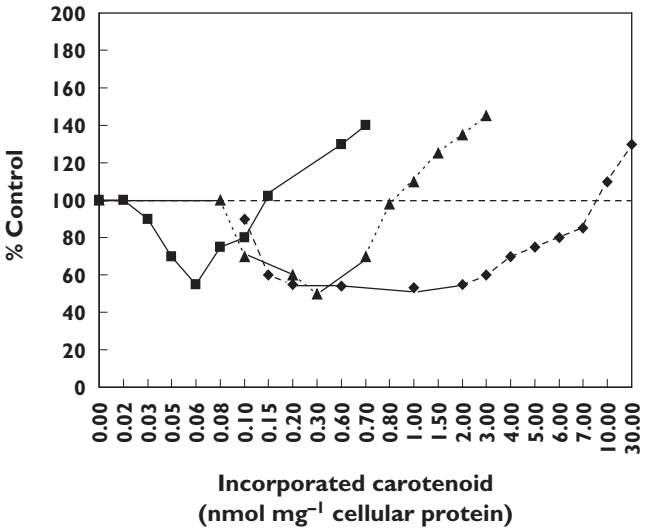

Figure 8

Effects of lycopene, beta-carotene, and lutein on UVB-induced TBARS formation in human skin fibroblasts [115]. Lycopene, (—■—); Beta-carotene, (- -◆- -); Lutein, (· · ▲· ·)

Skin

UV light causes photo-oxidative reactions that damage the functional integrity of sub-cellular structures, cells and tissues, especially in the skin and eye. The mechanism by which UV induces skin cancer, premature ageing of skin and various opththalmological diseases such as cataracts and age-related macular degeneration involve the formation of reactive oxygen species. Within this context it is known that low level carotenoid ingestion increases the risk of cancer, aged-related macular degeneration, cataracts and CVD [103–105]. Experimental studies have also indicated that UV-induced skin damage can be reduced by carotenoid supplementation [106–109], findings generally attributed to their antioxidant activities.

Despite their antioxidant properties, carotinoids also display pro-oxidant effects [110–113] as judged by biomarkers of lipid per-oxidation. Pro-oxidant effects have been proposed to account for an enhanced risk of lung cancer observed in epidemiological studies with β-carotene. [104, 105, 114]. These studies revealed an incidence of lung cancer that was about 20% greater than control values when β-carotene was given in high doses for a prolonged period to those with an enhanced risk of this disease.

Studies with cultured human skin fibroblasts were undertaken to explore the capacity of carotenoids (i.e. lycopene, β-carotene, and lutein delivered to cells using liposomes) to show antioxidant and pro-oxidant effects following exposure to UV [115]. The cells were exposed to UV light for 20 min that increased MDA values from a background of 0.4 nmol to about 1.2 nmol. The three carotinoids decreased the UV MDA values at low concentrations while increasing MDA formation as the concentrations increased. While the magnitude of the decrease in MDA production was similar for each agent, the range of the protective responses was variable. Even though this was an *in vitro* study, the carotinoid concentrations in human tissues are in the range used in this study (Figure 8).

While each carotinoid displayed the J-shaped dose–response there are notable differences with respect to the concentrations of carotinoids where the protection is initiated, ceases and where toxicity begins. These authors suggested that there are optimum levels of protection *in vivo*, which would like vary by individual.

Minoxidil and human keratinocytes

While attempts to grow hair have had a long history, a major breakthrough occurred in 1980 when Zappacosta [116] reported that a systemic anti-hypertensive agent, minoxidil, enhanced the growth of hair in patients. Over the next three decades minoxidil emerged as the most widely used drug for the treatment of androgenetic alopecia (AGA). While the mechanism(s) by which minoxidil increases hair growth remain to be elucidated more fully, the general emerging perspective suggests that it involves the restoration of normal keratinocyte proliferation [117, 118] via the regulation of calcium channels by its metabolite minoxidil sulfate. Particularly comprehensive in this regard have been the studies of Boyera *et al*. [117] involving a systematic investigation of the effects of minoxidil on human keratinocytes from different donors using different biological sources (i.e. interfollicular keratinocytes, follicular keratinocytes from microdissected hairs or plucked

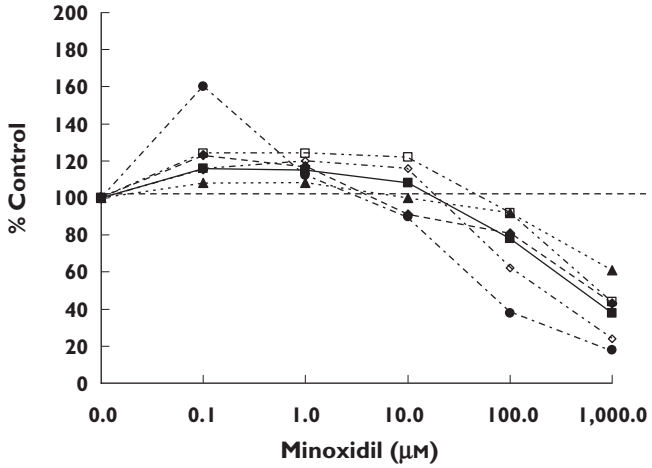

Figure 9

Effect of Minoxidil on normal human keratinocytes [117]. 1st Passage Epidemal NHK – high, (—■—); 2nd Passage Epidemal NHK – low, (-·◆-·); 1st Passage Follicular NHK – high microdissected, (··▲··); 1st Passage Follicular NHK – High plucked, (-·●-); Follicular NHK – Low, (-·⊡··); NHK Proliferation, (-·◇··)

hairs), a range of experimental conditions (e.g. high- and low-calcium medium, with or without serum, high or low epidermal growth factor concentration) using multiple but complementary endpoints to assess cellular proliferation (i.e. mitochondrial dehydrogenase activity, BrdU incorporation, lysosome content, protein content, lactate dehydrogenase released, and involucrin expression). Regardless of the specific experimental conditions assessed, minoxidil induced biphasic concentration responses with stimulatory effects at low (i.e. micro-molar concentration) and anti-proliferative, pro-differentiative and partial cytotoxic effects at higher concentrations (i.e. millimolar concentrations). Figure 9 illustrates this general relationship in a comparison of mitochrondrial dehydrogenase activities (i.e. biomarker for the number of viable cells) relative to controls across a range of experimental systems. Similar qualitative and quantitative concentration relationships also were reported for BrdU incorporation (i.e. marker of proliferative cells in S phase), neutral red (i.e. marker for the number of viable cells) and BioRad protein (i.e. biomarker for the total number of cells). The high degree of consistency of the concentration–response relationships for complementary endpoints strongly supported the overall reliability of the findings. The concentration–response relationships generally indicated that the maximum stimulatory responses regardless of endpoint were modest, being in the 15–30% range greater than the controls. The stimulatory concentration range is also consistent across endpoints and experimental systems, being approximately 10- to 100-fold.

The quantitative findings of the *in vitro* investigations of Boyera *et al*. [117] are remarkably consistent across the broad range of experimental protocols and suggest the possibility of providing mechanistic insight of clinical findings. In fact, the maximum plasma concentrations of minoxidil with a hair growth promoting dose is approximately 0.775 μM, a concentration seen to consistently induce stimulatory cell proliferation responses in the Boyera *et al*. [117] study. Therefore, the minoxidil treatment at low concentrations would be expected to maintain keratinocyte proliferation in conditions such as AGA while concomitantly preventing premature commitment of cells to differentiative pathways. However, Boyera *et al*. [117] raised the possibility that minoxidil may accumulate in some follicle compartments, such as the hair shaft which has particularly high concentrations of keratins and melanins. Such millimolar concentrations of the drug could occur adjacent to the keratogenic zone, thereby supporting keratinocyte differentiation along with hair shaft thickening.

Bone

Bisphosphonates

Osteoporosis is a major public health issue for ageing women. In the US alone nearly 10 million women over 50 years of age have osteoporosis with nearly double that many being at risk because they have low bone mass [119]. Bisphosphonates can prevent bone resorption and therefore have been employed in the treatment of osteoporosis. Bisphosphonates are synthetic analogues of pyrophosphate where a carbon atom replaces the oxygen at the center of the pyrophosphate. This chemical substitution results in the bisphosphonate becoming resistant to hydrolysis. It permits two additional side chains (R_1, R_2) of potential variable structure. The R_1 side chain contains an hydroxyl moiety leading to high affinity for calcium crystals and bone mineral. The chemical differences at R_2 have been exploited for the development of bone antiresorptive potency [120, 121]. Despite their successful chemical applications, mechanistic understanding of bisphosphonates has emphasized their direct inhibitory effects on mature osteoclasts [122, 123]. However, in addition to the inhibitory effects on osteoclastic bone resorption, Giuliani *et al*. [124] explored whether the chemical effectiveness of these agents may result from a direct effect on osteoblasts, thereby representing an alternative target for bisphosphonate-induced beneficial effects on the bone formation process. In their investigation Giuliani *et al*. [124] evaluated potential effects of etidronate and alendronate on the formation of early and late osteoblastic cell precursors by measuring the number of colony-forming units for fibroblasts (CFU-F) and colony-forming units for osteoblasts (CFU-OB) in murine and human bone marrow cultures. Using aspirates from the femurs of three-month old Swiss-Webster female mice, 10 to 12 treatment concentrations over nine orders of magnitude were employed along with controls. In the mouse marrow cultures, etidronate

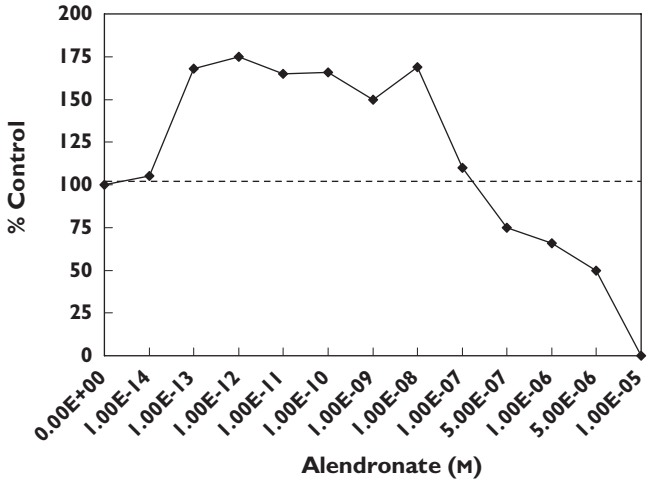

Figure 10

Effects of alendronate on CFU-F formation in murine bone marrow cells [124]

(10^{-5}–10^{-9} mol l^{-1}) enhanced the formation of CFU-F by 50–100% more than the controls. The alendronate treatment displayed a biphasic effect with stimulation occurring below 10^{-7} M with inhibitory responses occurring at higher concentrations. The maximum stimulatory response was 78% greater than controls. In the case of CFU-OB (i.e. mineralized nodule formation) both compounds displayed biphasic concentration responses with stimulation occurring at low, inhibition at higher concentrations. A comparable concentration–response relationship was also reported for CFU-OB in human bone marrow cells following alendronate treatment. The two lowest concentrations of alendronate which were effective in the formation of the early osteoblast precursors in this in vitro study were generally equivalent to those doses (5–10 mg day^{-1}) employed to treat patients with osteoporosis [125, 126].

In addition to the *in vitro* testing the authors also assessed the effects of both agents on CFU-F formation in young (3 months) and old (18 months) mice. There was good agreement between the *in vitro* and *in vivo* studies with low doses being stimulatory for both agents and each age group [124].

The mechanisms by these agents enhance the formation of early and late osteoblast precursors suggest an involvement with basic fibroblast growth factor (bFGF-α) [124]. The production of bFGF-α, a powerful mitogen for mesenchymal cells, increased by 50% with alendronate treatment over the same concentration range (10^{-8}–10^{-12} mol l^{-1}) capable of stimulating osteoblastogenesis *in vitro* (Figure 10) [127]. bFGF-α stimulates the proliferation of CFU-F and the formation of mineralization nodules in both animal and human cultures [128–132]. These findings suggest that bisphosphonates are most likely affecting an increase in the number of mesenchymal bone marrow cells committed to the osteoblast phenotype. Thus, the overall effectiveness of these compounds may represent a beneficial influence of osteoblast precursors to complement the previously recognized high dose inhibitory effect on osteoblastic bone resorption.

Since the ground breaking study of Giuliani *et al*. [124], a series of independent investigations have confirmed their basic conclusions, while generalizing the bisphosphonate induced biphasic dose–response on osteoblasts to a broader array of compounds, experimental models and endpoints. The quantitative features of the dose–response were remarkably consistent, including not only the magnitude and width of the stimulatory response but also absolute tissue sensitivity. That is, the response range for the CFU-F and CFU-OB for the female Swiss-Webster mouse bone marrow cells was generally consistent with MG-63 human osteoblast [133] and human osteoprotegerin production [134]. While the consistency in the quantitative features of the hormetic dose–response was not unexpected, the striking similarities in the absolute sensitivity across tissues to a range of bisphosphonates was unexpected and has not been previously discussed. Furthermore, the stimulatory range over which the osteoblast-proliferation occurs is extraordinary, being well over a 10 million-fold, and likely even wider. No mechanistic foundation to account for the broad stimulatory range has been offered (Figure 10).

Of relevance to the above assessment of bisphosphonate-induced biphasic dose–responses is that the molecular mechanism of the biphosphonates differ between their two structure subgroups, that is, those with single substrates (e.g. H, OH, Cl, CH_5) and lacking nitrogenation, and those with common substituents being a hydroxyl group together with a nitrogen-containing aliphate side chain or heterocyclic rings. Non-nitrogen compounds such as etidronate appear to form cytotoxic ATP-analogues following interactions. The nitrogen-based compounds inhibit farneyl diphosphosphate synthase, an enzyme of the mevalonate pathway, thereby reducing the prenylation of small GTP-binding proteins needed for normal cell function and survival. However, despite such marked differences in mechanism both groups biphasically induced osteoblast proliferation, suggesting other mechanisms that are shared [120].

A further possible mechanism by which nitrogen containing bisphosphonates may prevent bone resorption is via an anti-angiogenesis process as reported by Wood *et al*. [135] with the drug zolerdronic acid (ZOL) using multiple endpoints. These authors noted the osteoclastic bone resorption depends upon efficient vascularization of the haemangiogenic endothelials cells. The Wood *et al*. [135] study also reported a biphasic effect of biphosphonates [e.g. ZOL, pamidronate (PAM)] on endothelial cell adhesion consistent with the quantitative features of the hormetic dose–response, thereby suggesting a more complex and integrative biological response.

Statins

In 1999 Mundy *et al*. [136] were the first to report that a number of statins, in particular simvastatin and lovastatin, stimulated bone formation following a subcutaneous injection over the murine calvaria. This same treatment enhanced the expression of BMP-2 mRNA in osteoblasts. Since these findings were confirmed and extended to bone diseases, such as osteoporosis [137, 138], Yazawa *et al*. [139] explored whether statins could affect the regeneration of periodontal cells that affect hard tissue regeneration. Using human periodontal pigment (PDL) cells these investigators observed the capacity of simvastatin to biphasically enhance the proliferation of PDL cells (24 h after treatment) *in vitro* as well as alkaline phosphatase (ALP) activity (7 days after treatment). Since the effects of simvastatin on ALP activity were prevented by mevalonate, it suggested that these effects were caused by the inhibition of the mevalonate pathway. Other published findings supported the earlier observations of the capacity of simvastatin to affect bone formation. That is, osteoblast differentiation and mineralization in MC3T3-E1 osteoblasts and bone marrow cells (*in vitro*) were induced by simvastatin [140–142].

From a clinical perspective simvastatin is taken orally in 5, 10, 20, 40 or 80 mg tablets, with 20–100 mg tablets yielding maximum plasma concentrations in the range of 4.4×10^{-8}–3.0×10^{-7} M [143]. According to Yazawa *et al*. [139] the optimal *in vitro* concentration appeared to correlate with the 20 mg tablet dose, although it is difficult to relate *in vitro* concentrations with dose to target tissue.

It is noteworthy that bisphosphonates stimulate the formation of osteoblasts and possibly do so via the inhibition of the mevalonate pathway, thus mechanistically linking bone stimulation in osteoblasts and in human PDL cells.

Ocular diseases and cell proliferation

The successful treatment of intraocular proliferative diseases, such as proliferative vitreopathy (PVR), progressive traumatic traction retinal detachment (PTTRD) and intraocular neovascularization remain problematic despite notable advances in intraocular microsurgery in the direct handling of vitreoretinal tissues. This is due to the excessive accumulation of fibrous vascular tissue within the eye. Surgical treatment alone has also had limitations in the treatment of a range of other disorders characterized by progressive conjunctival or extraocular cicatrization including ocular pemphigoid (i.e. blisters), restrictive motility syndromes, and aphakic (lack of a natural lens) or neovascular glaucoma filtration surgery. According to Blumenkranz *et al*. [144], the central underlying feature that is common to these apparently unrelated clinical disorders is the rapid and uncontrolled proliferation of non-neoplastic cells within or about the eye. In such ocular-related proliferative disorders, the cells may be of diverse origin, including the retinal pigment epithelium, astroglia, macrophages, vascular endothelium, myoblasts, myofibroblasts, or fibroblasts. Nonetheless, the induced damage is generally accepted as being due to fibrocellular proliferation, active contraction of cellular membranes, and the formation and cross-linkage of newly formed collagen by fibroblasts and myofibroblasts. Such observations suggested that non-toxic pharmacological agents that inhibit the growth of rapidly proliferating cells may have value in the treatment of such diseases. Such reasoning led to a substantial series of cell culture investigations to find appropriate drugs which would be able to inhibit the proliferative response while not being toxic to ocular tissue. Other notable considerations included the toxicity of carrier solvents as well as the pharmacokinetics of the agent that might affect the periodicity of re-treatment.

In his 1988 reflective essay, Machemer [145] noted that he first thought to supplement surgical advances in the treatment of proliferative vitreopathy with pharmacological agents in the early 1980s with a consideration of the gout medication, colchicine, because of its capacity to be an inhibitor of mitosis. However, the selection of this agent for clinical application was problematic since clinical doses used for gout were far too low to affect cellular proliferation whereas higher doses were thought to markedly increase the risk of inducing toxicity in the neural tissue of the eye. This led to consideration of cancer chemotherapeutic agents, such as 5FU and daunomycin, since they were strongly anti-proliferative. While Machemer had concerns about their use due to toxicity, others proceeded to establish their therapeutic potential. Nonetheless, Machemer became interested in the possible use of corticosteroids since they had been reported to inhibit mitosis [146, 147] while having a very low likelihood of causing ocular toxicity. These collective perspectives lead to follow up investigations with a wide range of steroids, non-steroidal anti-inflammatory agents, as well as antimetabolites and potent biopeptides.

An evaluation was undertaken of such agents in which a broad concentration range was assessed, including concentrations below the proliferation inhibition threshold. This evaluation revealed that hormetic-like biphasic dose–responses commonly occur and that these responses were independent of the chemical agent and the biological system employed. The maximum stimulatory responses were modest, generally being only 30–60% greater than the control value. However, there was considerable variation in the width of the stimulatory concentration range. For example, in the case of DEX, Blumenkranz *et al*. [144] reported a low concentration stimulatory response across greater than five orders of magnitude whereas the fluoridated pyrimidines displayed a stimulatory response of approximately only 10-fold [148]. The causes of such inherent variability in the width of low dose stimulatory

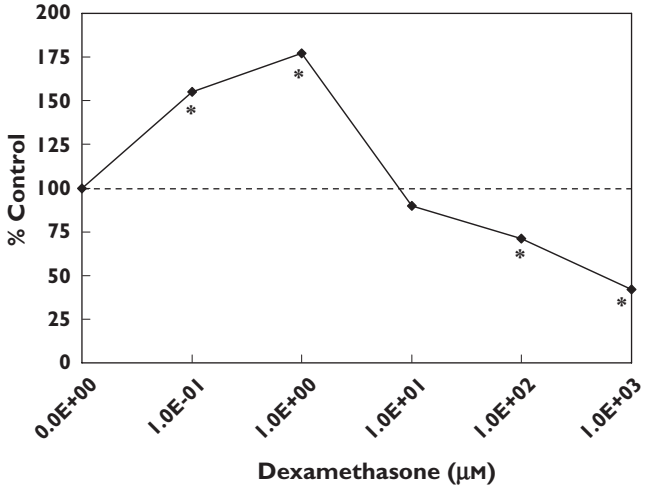

Figure 11

Effects of dexamethasone on cell growth and viability of cultured human RPE. *Significantly different from controls at $P \leq 0.05$ [210]

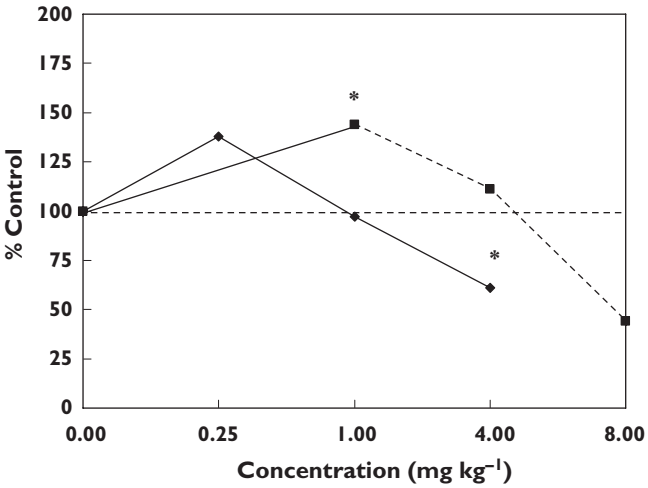

Figure 12

Effects of yohimbine and idazoxan on erections exhibited by rats in the reflex test. *Significantly different from controls at $P \leq 0.05$ [153]. Yohimbine, (—◆—); Idazoxan, (- -■- -)

response have not been addressed. The occurrence of inter-individual variation in low dose proliferative stimulatory responses of cultured retinal pigment epithelium proliferation was reported in by Yang & Cousins [149]. They noted that concentrations that were stimulatory for one individual might be inhibitory for another.

The implications of the inter-chemical and inter-individual variation in low dose stimulatory responses may have important implications. The ideal pharmacological agent would inhibit cell proliferation without being toxic and without being stimulatory in the low concentration zone. Investigators have yet to address explicitly the biomedical implications of a 30–60% stimulatory response over a certain number of days. Agents such as DEX could have a highly variable stimulatory zone depending on the individual and cell type. Even in the case of agents which have not shown the type of low dose stimulatory response as reported for DEX or trimicolon, these have not been typically well studied in the low dose zone. However, there is considerable evidence that these antimetabolites can be very effective in enhancing cell proliferation in the low dose zone [150]. The potential of pharmacological therapeutic agents such as DEX (Figure 11) to stimulate cell proliferation in ocular tissue at low doses may be a risk factor that needs to be considered in the treatment of surgical patients.

Male sexual behaviour

Considerable pharmaceutical interest has been directed toward improving aspects of male sexual behaviour, with particular emphasis on erectile dysfunction (ED). Despite the rapid commercialization of products over the past

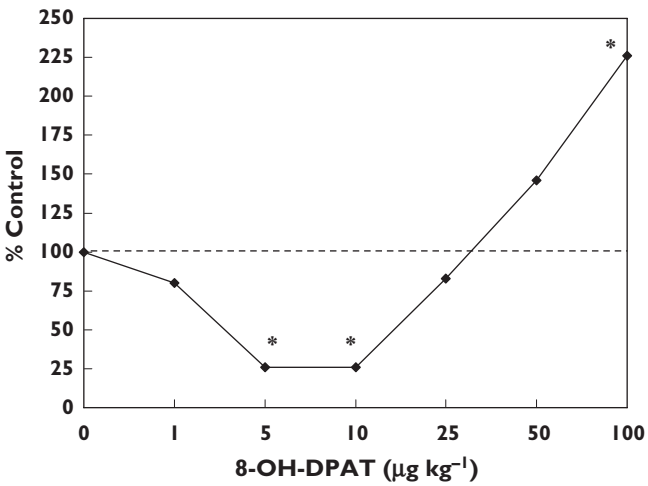

Figure 13

Effects of yohimbine on ejaculation latency of male rhesus monkeys. *Significantly different from controls at $p \leq 0.05$ [151]

decade, research on endogenous and exogenous agents that could enhance male sexual performance extends back to the 1960s. Of particular interest is that essentially all chemical groups that have been shown to enhance male sexual performance (e.g. penile erection, ejaculatory functions) have generally been shown to display hormetic-like biphasic dose–response relationships [151–156] (Figures 12 and 13). There are a sizeable number of such agents affecting various receptor systems, with particular research interest having been directed to dopamine, α-adrenergic agents, serotonin, opioids, nitric oxide, cholinergic agents, histamine, prostaglandin and oxytocin, amongst others.

An evaluation of the pharmacological foundations of each of these general areas of research concerning male sexual behaviour have typically utilized male rats of multiple strains with particular emphasis on the use of Wistar and Sprague-Dawley rats, with dogs being employed as the second most common animal model. The investigators have tended to use penile erection as the most commonly used general endpoint in studies with rats. Penile erection has generally been studied using the spontaneous erection model in which drugs are tested for their capacity to increase the incidence of erections as compared to non-stimulated male, that is, without the influence of a female. A relatively low proportion of studies dealing with the penile erection have used a penile reflex test to estimate the frequency of erections. Copulatory investigations have also been common with multiple endpoints being assessed including mounting behavior, intromissions and ejaculatory parameters. Particular applications in the research have been directed to castrated males or other models of dysfunction. In the case of the dog model the principal endpoint assessed has been ejaculatory performance. Limited studies with large numbers of doses have been published using non-human primates.

The receptor systems that have been assessed most extensively with respect to detailed dose–response evaluation and male sexual behaviour have been dopamine [157–167], α_2-adrenoceptor antagonists [152–154, 168–171], and serotonin [151, 172–179]. Each has a unique data base and scientific foundation that reflects investigator goals and research strategies. For example, in the case dopamine there has been extensive investigation of the dopamine agonist apomorphine, its metabolites, and other agonists. Considerable attention has been directed to clarification of the receptor subtype(s) that mediated the enhanced male sexual performance. Thus, extensive research has involved agonists and antagonists for receptor subtypes D1–D5 [164]. Similarly, there has been considerable research dealing with receptor interactions and the implications that specific agonists often display differential affinities for multiple receptors, each potentially affecting the sexual behaviour endpoint of interest. The case of dopamine has been generally similar for other receptor systems.

Since this general area of research was initiated in the 1960s there has been a progressive and, at times, rapid re-assessment of receptor pathways involved in the stimulation of male sexual behaviour. For example, each major receptor area has displayed a notable increase in the number of receptor subtypes, sometimes requiring the reclassification of agonist affinity to a newly discovered subtype with a consequent re-assessment of the stimulatory pathway. Likewise, numerous stimulatory agonists have been found to have affinities within multiple receptor systems after the initial findings that classified the agent as stimulating male sexual behaviour via a specific pathway. Despite this progressive growth in complexity of pathway interactions and signal convergences, the dose–response features have continued to reflect that of the hormetic-like biphasic dose–response.

Even though essentially all papers with evidence of a biphasic dose–response relationship for a male sexual behaviour have acknowledged their occurrence, very few such papers have provided even speculative hypotheses that might account for such common and reproducible observations. Several have been provided in areas such as with dopamine [159] and α_2-adrenoceptor antagonists [152] but even these were put forth as speculative. Most of the detailed focus was directed to trying to clarify pathway involvement. In addition to the above major research areas, there have been reports published using botanically derived agents [180] which also have commonly displayed the hormetic-like biphasic dose–response relationships.

Of particular current interest have been the phosphodiesterase 5 inhibitors, which include the commercial products of Viagra, Cialis and Levitra. However, perhaps because of the history of their discoveries which are principally within the pharmaceutical industrial setting there has not been the same type of extensive dose–response studies to evaluate in the open literature. However, in the case of sildenafil recent studies have indicated that it displays hormetic-like biphasic dose–response relationships for human sperm motility [181] and in animal model studies concerning memory performance [182].

The dose–response features have generally conformed to that of the hormetic dose–response model with respect to the maximum stimulatory response and the width of the stimulatory range. However, in the case of spontaneous penile erection it is not uncommon to observe a four-fold increase in erections. While this is nearly double what might be expected, it may be an artifact of the use of a spontaneous rat model in which the control response rate is very low. There were also interspecies differences in the width of the stimulatory responses. For example, research assessing the effects of yohimbine in the rat model indicated that the width of the stimulatory response was very narrow, implying that this was due to its non-selective α_2-adrenoceptor agonist binding [170]. However, research with the beagle dog model for ejaculatory endpoints revealed a considerably greater width of the stimulatory response which exceeded 30-fold [154].

Prion diseases, protein folding and hormetic dose–responses

Prion diseases have been shown to occur via both inherited and infectious processes, with infectious human prion diseases comprising only about 1% despite their notoriety [183]. Considerable evidence now exists which indicates that prions are comprised of a misfolded prion protein (PrP) isoform (PrP^{Sc}) of a glycolipid-anchored host protein

(PrP^C). While prions lack nucleic acid, their diversity is related to the conformation of PrP^{Sc} [184].

Prion diseases are progressive neurological disorders found in animals and humans. While rare, they are invariably fatal. Prion diseases have been of particular societal concern since there is the potential for cross species transmission of the scrapie condition (named from the symptoms of the condition due to compulsive scraping off of the fleece) from sheep to cattle where it has produced bovine spongiform encephalopathy (BSE) [185]. Hundreds of thousands of BSE-affected cattle have been slaughtered in order to prevent this epidemic in cattle and to protect the human food supply. As a result of public health concerns with this and other prion disease conditions, substantial efforts have been initiated to discover drugs effective in preventing the onset and/or progression of the disease process.

The normal cellular prion protein PrP^c is relatively susceptible to proteolysis compared with an abnormal isoform PfP^{Sc}. PrP^c may be converted to PrP^{Sc} and this process is considered a biochemical correlate of agent replication in cell culture and animals with the prion disease. According to Rudyk *et al*. [186, 187], one therapeutic strategy in the treatment of prion diseases is the discovery and use of agents that slow the replication of the infectious agent and delay the occurrence of the clinical disease when administered directly into the brain or peritoneum at or near the time of infection in the animal model. Using the non-neuronal scrapie infected mouse cell line model (SMB) originally cloned from an infected mouse brain [188], these authors assessed the capacity of Congo red and a number of its structural analogues to prevent the formation of PrP-res, a biomarker (protease resistant prion protein) of the infectious agent in this cell line. Congo red was selected because of its well recognized affinity for amyloid protein, its capacity to inhibit the replication of prion agents and thereby prevent the accumulation of PrP^{Sc} [186], and its ability to prolong the survival time of hamsters infected intraperitoneally with two different strains of scrapie [189]. However, Congo red is limited by its poor capacity to pass the blood brain barrier and that it is metabolized to benzidine, a human carcinogen. These factors lead to a broader assessment of structural analogues with improved penetration of the blood brain barrier and whose metabolites would be non-carcinogenic. In an initial follow up dose–response assessment 18 agents were tested including Congo red using only two concentrations (100 and 1 μM). While the 100 μM concentration markedly prevented the formation of PrP^{Sc} there was the general observation of an enhancement of PrP^{Sc} formation at the 1 μM concentration by numerous sulphonic acid derivatives of Congo red but not with the two cases of carboxylate derivatives. The increase of PrP-res formation ranged from approximately 140% to 225% (relative to a control value of 100%) depending on the specific analogue tested. In follow-up experiments selected agents were tested over six concentrations with log concentration spacing. Not only was the low concentration stimulation response confirmed in representative sulphonic acid derivative compounds, but even Congo red (Figure 14), which was negative in the preliminary studies with only the two concentrations (1 and 100 μM), was positive once the bioassay included even lower concentrations [186].

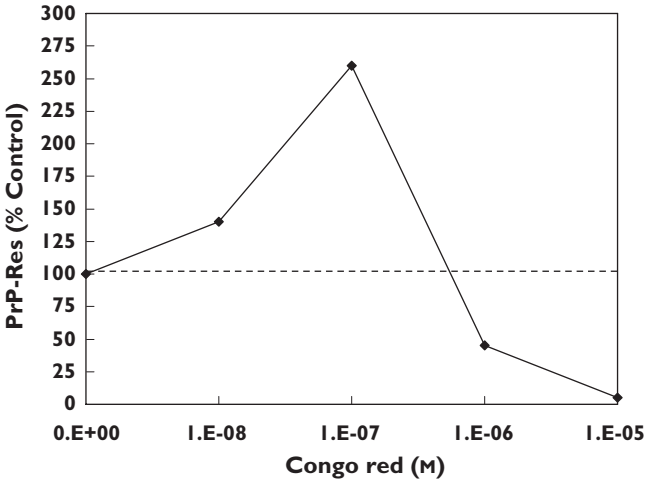

Figure 14
Effects of Congo red in the SMB cell assay [186]

These dose–response features of Congo red and its sulphonic acid derivatives are consistent with the quantitative characteristics of the hormetic dose–response model. These findings are of medical concern because they raise the possibility that such potential therapeutic agents may have the possibility of enhancing the occurrence of the prion disease within the low concentration range.

The mechanism by which such low concentration related increases in PrP^{Sc} may occur have been addressed to a limited extent. The low concentration stimulatory response was hypothesized by Rudyk *et al*. [186] to be related to an observation by Caspi *et al*. [190] that Congo red inhibited new PrP^{Sc} synthesis and PrP^{Sc} degradation in scrapie-infected neuroblastoma cells. Rudyk *et al*. [186] also speculated that these compounds, as monomers at low concentrations, may stimulate PrP^{Sc} formation due to binding to PrP^C at just one site, while at higher concentrations in physiological salt, they may form a supramolecular ligand, a liquid crystal, which binds PrP^{Sc} and/or PrP^C, preventing their interaction [191, 192].

Discussion

The paper asserts that the hormetic dose–response is more common and fundamental than other dose–response models, including the long revered threshold model, far out-competing them in fair head-to-head evaluations. It is argued further that we are in the midst of a major dose–

response revolution that has highly significant implications for essentially all branches of science that are concerned with dose–response relationships and adaptive responses.

The last three decades have witnessed growing interdisciplinary evidence of hormetic-biphasic dose–responses that are characterized by remarkably similar quantitative features of the dose–response and similar underlying mechanistic explanatory strategies. It is the emergence and integration of these findings from diverse biomedical fields that has lead to the consolidation of the hormesis dose–response concept and motivation to re-discover the historical foundations of the dose–response [193–197].

The concept of hormesis can also be considered within a preventive context. Considerable research has associated beneficial responses from low to moderate levels of exercise with hormetic mechanisms [198–201]. Likewise, the benefits of caloric restriction [202, 203] or certain fasting regimes [204, 205] have also been proposed as manifestations of hormetic effects. These developments are believed to have the potential to enhance the quality of life within ageing populations [206].

This paper presented a spectrum of examples in which hormesis is having or could have an important role in clinical pharmacology. The examples selected are illustrative of the potential of the hormesis to affect the biomedical sciences and to improve its capacity not only to enhance human health and performance but also to avoid harm in patient treatments. A key point is that the concept of hormetic-biphasic dose–responses is already an important feature within clinical pharmacology, especially within the areas of drug discovery for anxiolytic drugs, anti-seizure drugs, memory, neuroprotection, drug addiction, pain, and others. In fact, hormetic-biphasic dose–responses are common to these fields, affect decisions on drug development but almost never is the term hormesis used to describe these versions of the dose–response relationship. The hormesis concept is also important in areas of clinical pharmacology in which high doses are important for killing harmful organisms or tumour cells. In these cases, there may be health concerns over the potential of hormetic-biphasic dose responses, such as tumour cell proliferation or prostate enlargement, at hormetically acting low concentrations. In these cases as well, the low dose stimulatory response has not been referred to as a hormetic dose–response. Yet it is argued that such cases are manifestations of the hormetic dose–response, reflecting its centrality within the biomedical sciences, its generalizability across biological models, gender and age groups, endpoints and chemical classes and the important constraints it imposes on the quantitative features of the dose–response as an indicator of biological plasticity. Recently a large number of biomedical scientists have proposed the use of a common terminology that more effectively places biphasic dose–responses and stress responses within a broad hormetic framework [214]. Finally, it is time for the hormetic dose–response to be integrated within the education and training of current and future biomedical scientists and to improve the design and conduct of studies affecting drug discovery and safety evaluation as well as providing a sound dose–response framework in the refining or fine-tuning of drug doses for patients in clinical settings.

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Hormesis is central to toxicology, pharmacology and risk assessment

Edward J Calabrese

Abstract

This paper summarizes numerous conceptual and experimental advances over the past two decades in the study of hormesis. Hormesis is now generally accepted as a real and reproducible biological phenomenon, being highly generalized and independent of biological model, endpoint measured and chemical class/physical stressor. The quantitative features of the hormetic dose response are generally highly consistent, regardless of the model and mechanism, and represent a quantitative index of biological plasticity at multiple levels of biological organization. The hormetic dose-response model has been demonstrated to make far more accurate predictions of responses in low dose zones than either the threshold or linear at low dose models. Numerous therapeutic agents widely used by humans are based on the hormetic dose response and its low dose stimulatory characteristics. It is expected that as low dose responses come to dominate toxicological research that risk assessment practices will incorporate hormetic concepts in the standard setting process.

Keywords

hormesis, hormetic, biphasic, U-shaped, adaptive response, inverted U-shaped

Introduction

This paper discusses insights that have been gained as a result of assessing the concept of hormesis since approximately 1990. Of the two dozen new findings and ideas that will be discussed in this paper, essentially all were unexpected. Of particular surprise was that prolonged and detailed assessment of the nature of the dose response, especially in the low dose zone, would provide important and basic conceptual insights that have relevance to all biological systems. Thus, while the plan was to assess hormesis, the journey has yielded far more than was anticipated. Each discovery/insight is briefly described and referenced. It is hoped that the reader will be intrigued by the range of biological insights that studying the hormesis concept has revealed. Furthermore, this paper will provide a useful and concise summary of the current status of hormesis-related research as well as insights into possible future developments.

Critical failure of public health regulatory agencies to validate the threshold dose-response model in the 20th century

The threshold dose-response model is fundamental to all aspects of biology that use dose-response relationships. This model has been central to toxicology, pharmacology and public health regulatory agencies since the 1930s, affecting chemical/drug safety evaluations, modern risk assessment practices and public health exposure standards. The study and application

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of the threshold dose-response model is therefore central to the fields of toxicology, pharmacology and risk assessment.[1-3]

This centrality of the threshold dose-response model within the biomedical sciences and public health regulatory agencies has lead to the assumption that this dose-response model has been studied in detail, scientifically vetted and validated, and can be reliably assumed to provide accurate estimates of biological responses especially in the low dose zone (i.e. below toxicological and pharmacological thresholds). In the course of our assessment of hormetic dose-response relationships, the question was raised as to whether the threshold dose response was formally assessed for its capacity to predict below threshold responses. While there was the general belief that it must have been, given the importance of this question and the universal acceptance of this model within the scientific and regulatory communities, our comprehensive attempts to find research that had addressed this issue uniformly failed. Yet, this failure was very unsettling, for how could the biomedical community have built an entire toxicological and drug testing and regulatory framework upon a dose-response model that had not been validated? This seemed to be implausible and therefore could not possibly be true. It most likely meant that our comprehensive attempts were not really 'comprehensive' and that we must have been missing the obvious. Yet, renewed attempts with differing search strategies to ferret out the scientific vetting of the threshold dose-response model continued to fail to yield any relevant publications. Eventually a disturbing conclusion was reached, that is, the principal dose-response model upon which chemical and drug toxicity testing has been based had never been validated, but simply accepted as true, being passed down with authoritative conclusionary statements from textbook to textbook, from professor to student, from regulatory agencies to citizens, across generations of scientists, creating an illusion of knowledge and informed guidance.

This situation led to two avenues of further inquiry. The first was the need to develop an historical reconstruction of the threshold dose-response concept that would have lead to how this 'blind' acceptance without validation and vetting occurred (see ref 4 for a detailed assessment). The second critical issue was the need to test predictions of the threshold dose-response model in large data sets using a priori entry and evaluative criteria.[5-9] That is, we would conduct our own vetting of the threshold dose-response model to make accurate predictions of responses below the threshold. These studies have documented that the threshold dose response very poorly predicts responses below the estimated threshold, a performance that was broadly generalizable. This failure of the threshold model to make accurate predictions of responses below the threshold in the above published data was also consistent with the publication of a large number of studies within the hormesis database[10,11] that are supportive of the hormesis dose response and not the threshold model.

These findings point to a critical and ongoing failure of the scientific and regulatory communities to properly validate models, especially ones that are directly used to affect public health and medical practices. The societal costs of the failure to vet and validate the threshold dose-response model for the past 75 years are unknown. However, one must ask how it was possible for U.S. federal agencies such as the EPA, FDA, ATSDR, NIEHS, NIOSH, OSHA and others to never conduct or fund studies that would have addressed this question. The same question may be asked of private sector funding of toxicological and pharmaceutical research and why this question has never been addressed.

It should be noted that the FDA did recognize the need to validate linearity at low dose predictions in the mid-1970s, with the mega-mouse testing of the carcinogen 2-AAF. However, this effort revealed that risks lower than 1/100 were not practically achievable for carcinogens within chronic animal bioassays. The failure of the study to adequately test linearity at low dose modeling, despite the use of enormous resources (e.g. 24,000 animals), lead to a continued reliance on non-validated models for risk assessment of chemical carcinogens. An important irony was that a detailed analysis of the FDA/2-AAF study by an expert panel of the US Society of Toxicology revealed an unequivocal hormetic dose response for bladder cancer, with risks decreasing below the control group at low doses.[12]

Hormesis: it is real and common

When the BELLE Advisory Committee was first organizing, there was no generally accepted position on what was the status of hormesis within the scientific community. However, there were considerable questions over whether it was a real, reproducible phenomenon. Its status within the scientific community in the late 1980s and early 1990s was marginal

at best. In fact, from 1945–1989, the Web of Science reports only 159 cumulative citations using the terms hormesis or hormetic, all appearing from 1982 onward. The hormesis concept had therefore been explored only to a very limited degree through the 1980s. In contrast, in the year 2009 alone, the number reached 2460. So, the question maybe asked as to how hormesis emerged from an uncertain and marginalized concept to one that became accepted as real?

The key initial activity derived from a desire of the Texas Institute for Advanced Chemical Technologies (TIACT) based at Texas A&M University to determine whether hormesis was real or not. More specially, Dr Paul Deisler, a board member of TIACT, wanted TIACT to fund a study to answer this question. His idea led to a grant being given to the University of Massachusetts in 1995. It was the TIACT funding that lead us to create objective evaluative criteria to assess the existence of hormetic dose responses and to the conclusion that hormesis was not only a real and reproducible phenomenon but that is was likely to be very general, being independent of biological model, endpoint measured and chemical class/physical stressor agent.[13] This research has continued to the present with a progressively expanding database of findings of hormetic dose responses.[10,11] Specialized studies have been published on numerous receptor systems,[14-22] chemotherapeutic agents,[23] ethanol,[24] inorganic agents,[25] immune responses,[26] human tumor cell lines,[27] numerous neuroscience endpoints,[28-41] plant biology[11] amongst others. These findings have added more support to the conclusion that the hormetic dose response is highly generalizable with broad-based applications.

Development of a frequency of hormesis

Even though the above discussed research indicated that hormesis was real and a very general phenomenon, it did not provide a measure of the frequency of hormesis in the toxicological and/or pharmacological literature. Estimating the frequency of hormesis was considered to be of importance for regulatory agencies. For example, different strategies or policies could be developed if the hormetic frequency was <5% versus >40%. Thus, just knowing that hormesis was a real biological phenomenon was insufficient. This lead to an evaluation of nearly 21,000 articles in three toxicology and/or pharmacology journals from their inception to the most recent, assessing all articles with a priori entry and evaluative criteria. It is interesting to note that only 2% of the dose responses satisfied the entry criteria but of those that did, nearly 40% satisfied the evaluative criteria for hormesis.[5] Thus, for the first time, there was documentation of a frequency of hormesis within the published literature.

Comparing the threshold, linearity at low dose and hormesis models: which is most frequent?

In general, our research has focused on comparing the hormetic dose response with the threshold dose response for frequency. This is because the endpoints that had been studied in the most appropriate manner (i.e. strongest study designs) have involved non-cancer endpoints. This fact has lead to giving the linear model less emphasis in our publications. In these comparisons, the most striking observation is that the threshold dose-response model consistently performs very poorly. This has been shown in multiple studies using a wide range of biological models, endpoints and agents.[5-9] In contrast, the hormetic model has performed very well in these same head-to-head comparisons. However, recently there has been the proposal that all agents may induce their toxic effects via a linear, non-threshold manner.[42] In our studies that are cited above in this section, it was found that the linear at low dose model, like the threshold dose-response model, performed very poorly in our evaluations, thereby not supporting this new attempt to generalize the linear model.

Defining hormesis

In a broad reading of the general or popularized articles on hormesis, it has often been defined as a low dose beneficial response to a stressor agent. However, Calabrese and Baldwin[43] proposed that the dose response definition of hormesis be decoupled from a decision on whether the response was beneficial or not. This was done because it had become obvious to us that the low dose hormetic stimulation could be either beneficial or harmful, depending on the situation. For example, an antibiotic such as streptomycin may stimulate the proliferation of harmful bacteria in an animal while killing the bacteria at higher doses. Thus, at low doses, the streptomycin would be helping the bacteria but harmful to the patient while the reverse would be the case at higher doses. A chemical

may be seen to display an enhancement of longevity at low doses but decreasing longevity at higher doses. However, whether the increase in longevity for the individual would be beneficial for the species may not be true. Thus, the decision on whether the low dose hormetic response is beneficial or not can be complex and not necessarily immediately obvious.

Quantitative features of the hormetic dose response

When we initiated research on hormetic dose responses, we did not provide overriding consideration to the quantitative features of the dose response. Our thinking was far more qualitative at the early stages of development, that is, was there a low dose stimulation and was it reproducible. However, once data emerged on several thousand hormetic dose responses that were assessed for various dose-response parameters, it became clear that the most consistent quantitative feature of the hormetic dose response was the magnitude of the stimulatory response. Rarely was it greater than twice the control group. In general, the maximum stimulation for hormetic responses appears to be 30%–60% greater than control group.[44] This feature was consistent across biological models, endpoints and agents tested. This was an important observation since it clarified why hormesis could be difficult to document. That is, since the maximum stimulation was modest, it would require the use of rigorous study designs along with considerable statistical power.

With respect to the width of the stimulatory response, this was generally modest as well, typically being about a factor of 10. However, in about 2% of the cases, the width of the stimulatory zone was quite wide, exceeding a factor of 1000.[3] These observations have considerable toxicological and clinical implications as one considers the therapeutic zone or zones of exposure to avoid.

Another feature of the hormetic dose response curve is that it was always adjacent to the threshold response. This characteristic would make the upper boundary of the hormetic response very predictable, a factor that could be of considerable value to those involved with risk assessment and therapeutics.

Is there a single mechanism for hormesis?

This has been a common question raised at various conferences held on the topic of hormesis. When one considers that the hormesis phenomenon is extremely general, being independent of biological model, endpoint, and chemical class, it quickly becomes clear that a single proximate mechanism is not possible to account for the diversity of hormetic dose responses. However, there appears to be a common overall strategy of resource allocation within all biological systems, regardless of endpoint measured. The hormetic dose response may quantify how the system allocates resources. This is reflected in the observation that the maximum stimulatory response is typically limited to only 30%–60% greater than the control group.

General hormetic mechanisms: direct stimulation and overcompensation stimulation

Another issue that was not considered in the early evaluative stages of the hormesis concept was whether it occurred via a direct stimulation or via compensatory response. However, this would become an important consideration as will be seen below. My first research experience introduced me to the concept of hormesis, but I was unaware of the term or its temporal qualities. I observed that a synthetic growth inhibitor consistently induced a biphasic dose response for growth in Peppermint with a low dose stimulation and a high dose inhibition.[45] Although plant growth was measured weekly, the results of greatest interest were those at the end of the study, which was typically about 6 weeks. More than two decades later, I read several papers by Tony Stebbing on hormesis that emphasized the importance of the dose-time response in assessing hormesis.[46] He indicated that initially there would be a disruption in homeostasis (i.e. toxicity), followed by an overcompensatory response that would be seen as a stimulation. This encouraged me to go back to my original laboratory notebooks, re-analyzing the data in the manner suggested by Stebbing. When this was completed, Stebbing's prediction was confirmed. That is, during the initial weeks of the study, there was a dose-dependent decrease in growth followed by the overcompensation growth stimulation.[47] This re-assessment was possible because the study design employed many doses and a repeated measures component. The majority of experiments do not include both components, thereby preventing a detailed dose-time response. In the hormesis database,[10,11] about 20% of experiments have a dose-time relationship. These experiments have been important in

clarifying that hormetic dose responses may occur via the overcompensation stimulation mechanism. However, we also observed that there were numerous reliable examples in which hormetic dose responses occurred as a result of a direct stimulation, with no initial disruption in homeostasis.

These observations were interesting because they indicated that hormesis could occur by two different modes of action. Despite this clear difference in mechanism, the quantitative features of hormetic dose responses were the same for the direct and the overcompensation stimulation types of hormesis. Since most studies demonstrating hormesis do not contain a time component, one is not able to know whether the particular case of hormesis is direct stimulation or overcompensation. The question was raised (and will be addressed later) as to why these two types of hormesis would also display the same quantitative features of the dose-response relationship even though they were affected via different mechanisms.

An hormetic mechanism strategy

A wide range of drugs has been found to reduce anxiety in rodents by activating one of a variety of specific receptor pathways. Regardless of the drug used and the pathway activated, the quantitative features of the dose responses are similar. Another interesting feature is that the co-administration of anti-anxiety drugs that act via different mechanisms (i.e. activate different receptor pathways leading to the decrease in anxiety), regardless of drug potency, have their combined responses limited by the constraints of the hormetic maxima (i.e. plasticity constraints). This suggests that there is a downstream integration of multiple pathways, each of which can facilitate a reduction in anxiety. This downstream integration/conversion suggests a type of carousel model in with the resulting molecular product, that is, the dose response (e.g. analogous to the speed of the carousel) being similar.

High risk groups

The issue of high risk groups and how they are protected by environmental health standards is an important public health consideration. In 2001, we were challenged by Lave[48] to explore this issue since our earlier publications of hormesis had been directed to other questions. In a 2002 paper, Calabrese and Baldwin[49] reported that hormetic dose responses were found to be generally independent of inherent susceptibility. The principal finding was that those at increased risk have their dose response shifted to the left, showing hormesis and toxicity at lower doses than the so-called normal segment of the population. However, in some cases, the susceptible segment of the population is at high risk precisely because it lacks the adaptive hormetic mechanism. Furthermore, the quantitative features of the dose response for those at increased risk are similar to the normal segment of the population. The knowledge of hormesis and differential susceptibility is important for those involved in setting environmental and occupational exposure standards as well as for the pharmaceutical industry, which may target the hormetic stimulation when defining the therapeutic zone or when the hormetic zone needs to be avoided due to toxicity concerns.

Toxicological/pharmacological potency

Agents can widely differ in their potency for producing the same endpoint. Such differences could exceed several orders of magnitude. However, despite such differences in potency, there is no difference amongst these agents with respect to the quantitative features of the hormetic dose response nor other qualities of the hormetic response.[3] This is an important concept since a very potent agent will display the same quantitative features of the hormetic dose response as a weak agent, but doing so at a far lower dose.

Mixtures and hormesis

Mixtures have not been extensively studied within an hormetic context. However, there are sufficient data published that permits one to make some tentative general conclusions on how they are handled within an hormetic framework.[50] Particularly insightful have been the studies of Flood and his colleagues[51-54] concerning the effects of drugs on memory in rodents. These investigators have consistently shown a complex dose-response relationship. Most importantly, the maximum extent to which they could increase memory was constrained by the so-called 30%–60% stimulation rule. This was the case regardless of whether one or multiple agents were administered. If two or more memory-enhancing drugs were administered, there could be an additive or greater than additive relationship, but this would have to occur at a very low dose, where the response was some distance below the 30%–60% physiological performance cap. As the response approaches the maximum, the nature of the interaction would change from greater

than to less than additive. In effect, the nature of the hormetic interaction is principally seen at the level of dose rather than response. These findings indicate that the stimulatory response will be limited to the 30%–60% zone but that it may be possible to achieve this response level with a considerably lower dose due to the chemical interaction. Flood indicated that this would reduce the likelihood of experiencing adverse side effects. The concept of mixture responses within an hormetic dose response context is considerably different than that which is typically studied within a toxicological framework. The hormetic interaction has important response constraints, whereas this is not the case for standard toxicity endpoints at doses greater than the threshold.

Hormesis: a quantitative index of biological plasticity

The most striking feature of hormesis is that the stimulatory response is consistently modest with the maximum response about 30%–60% greater than the control value. Since this is the case regardless of mechanism, endpoint and model, pharmacological potency, for mixture responses and for chemical class, it strongly suggests that this response describes the plasticity of biological systems at multiple levels of organization ranging from the cell to the organ to the organism.[55,56] The findings indicate that this biological response is highly conserved as it is seen from organisms ranging from bacteria to man as well as in plants. These findings have important implications for clinical therapeutics as well as all dimensions of biological performance.

Preconditioning is a manifestation of hormesis

The term preconditioning entered the medical lexicon in 1986 when Murry et al.[57] reported that a brief occlusion of the coronary artery of dogs 1 day prior to inducing a major myocardial infarction reduced cardiac damage by about 80% as compared to the control group in which only the myocardial infarction was induced. These findings initiated a cascade of research, which was generalized well beyond the cardiac system, yielding similar protective findings. While most of these studies used only one or two types of exposures making it impossible to assess an hormetic explanation, a number of studies have teased out the dose response of the conditioning agent/exposure regiment.[58,59] In these studies, the conditioning agent displays an hormetic biphasic dose response, with similar quantitative features of hormesis. The findings clearly indicate that there is an exposure optima with the protection dropping off on either side. If the preconditioning exposure is too high, then it could further enhance the toxicity of the subsequent toxic or harmful exposure/treatment.

Hormesis and the 21st century

In an earlier question/answer, it was noted that the vast majority of papers reporting hormetic dose responses are recent, occurring since the year 2000. One major reason for this is that in the mid-1980s, there was a major shift toward the use of cell culture and the study of cell lines. The use of cell cultures often has employed 96 cell plates that allow for the assessment of 7–11 concentrations in each experiment. This is 2–3 times more treatment groups than the typical in vivo rodent assay. This was what the hormesis concept required in order to increase the likelihood of it being observed. In 2007, the US National Academy of Sciences (NAS)[60] published a book concerning toxicity testing for the 21st century. Amongst their far reaching recommendations was the eventual elimination of the chronic bioassay and its replacement with well-validated in vitro studies using various human cell lines. If these recommendations are followed it suggests that hormetic dose responses will be a central feature of 21st century toxicological findings[60] as in vitro studies will often employ a larger number of treatment groups across a broader concentration range than would occur with a traditional in vivo toxicological study.

Hormesis and biological performance

The hormetic low dose stimulatory response represents a new concept in toxicology and pharmacology, being a measure of biological performance. This is seen with respect to endpoints such as plant growth, strengthening bones, improving memory, decreasing anxiety, increasing seizure thresholds, growing hair, attracting neutrophils to sites of infection, decreasing mutation rate and tumor formation and many other responses. The dose response therefore has two response components, that is, the above the threshold response and the below the threshold response. The above threshold response is generally unrestrained as seen with high dose toxicology in which evidence of tissue damage or mutational effects or other toxic

endpoints can increase by several hundred or even a thousand or more fold. While there are often pharmacokinetic limits on the induction of toxicity, toxic responses are generally very progressive and have the potential to massively increase. This is not the case with responses below the threshold where the hormetic stimulation becomes manifest.

Drug benefit limitations

When a new and improved drug reaches the market, there maybe the assumption that it will produce a greater benefit than older competitive drugs. It will grow more hair, reduce anxiety better, make stronger bones and boost memory. The hormesis concept indicates that this is not necessarily the case. Hormesis imparts a limit on how much gain there is in the biological system. Many hundreds of endpoints display the same approximate level of modest maximum gain, that is, only in the 30%–60% range. Even the vastly more potent drugs will not increase the performance. They simply give the same performance, but at a lower dose. The gain in the system is limited by the constraints imposed by plasticity.

Is hormesis related to homeopathy?

In earlier writings, I have separated hormesis from homeopathy. I even went so far as to say that homeopathy was the equivalent of a scarlet letter on the forehead of hormesis.[61] The lay public and even many in the medical profession often confusedly merged the concepts. Hugo Schulz discovered the basic concept of hormesis in the mid-1880s in experiments assessing the effects of disinfectants on the metabolism of yeast. Through a type of convoluted logic, Schulz came to believe that he had discovered the explanatory principle of homeopathy. In fact, the studies of Schulz had nothing to do with the concept of homeopathy. However, biomedical investigators in The Netherlands[62,63] have tried to explicitly design studies that might link the two concepts via what is now called postconditioning hormesis.[64] These investigators demonstrated that low doses of heat or chemical toxin when given after a stress (i.e., disease process simulation) can amplify the initial response to stress in a hormetic-like fashion. While this research was experimental rather than clinical, it provides a framework for further study. Given legitimate criticisms of the ultra dilutionist wing of homeopathy, it must be emphasized that this research of Van Wijk deals with exposure to stressor agents that can be readily measured and is fully capable of being evaluated within normal biomedical experimental protocols. Unfortunately, this research was published during the mid-to-late 1990s and has not been continued. Nonetheless, this new experimental framework provides a conceptual vehicle to facilitate the evaluation of some homeopathic treatment strategies within an hormetic context.

Hormesis and harmful effects

When I first started to assess hormetic dose responses, little thought was given to the possibility that harmful effects would occur. Most attention was given to whether hormesis was a real, reproducible phenomenon. However, it eventually emerged that the low dose stimulatory hormetic responses could at times lead to undesirable effects. For example, low doses of antibiotics were shown to occur as early as the mid-1940s by FDA researchers to stimulate the proliferation of harmful bacteria. In vivo studies with low doses of penicillin as well as streptomycin enhanced mortality in mice given an LD_{50} dose of a deadly bacterial strain while preventing death at higher doses.[65,66] This remains a potentially very significant area of public health research.

Low doses of numerous agents, including anti-tumor drugs, have been shown to enhance the proliferation of tumor cells.[27] These findings suggest that under certain conditions, the administration of anti-tumor drugs to cancer patients may enhance the proliferation of the tumor cells. This is particularly the case for drugs with a long biological half-life. Some anti-tumor drugs used for the treatment of humans, such as the drug suramin, not only display the hormetic biphasic dose response with multiple tumor cell types but also have a rather prolonged period of residence within the human body, taking nearly 2 months to clear.[67] In such cases, there would be a prolonged period of time during which the drug would be present at very low concentrations. Whether these concentrations would be optimized to enhance tumor cell proliferation is an important question to resolve. The fact that anti-tumor agents can stimulate tumor cell proliferation at low doses within an hormetic context has generally not been widely appreciated by the cancer treatment community that emphasizes the high-dose killing portion of the dose-response curve.

This concept has been generalized to other areas of cancer treatment, including brain tumors. For example, anti-inflammatory agents such as dexamethesone

have been shown to enhance the proliferation of human neuroepithelial brain cancer cells in vitro displaying an hormetic dose response.[68-71] Such findings generated considerable concern amongst brain surgeons who commonly used anti-inflammatory agents in the management of their patients' pain.

Another potential adverse effect caused by the low dose hormetic stimulation may include the enlargement of the prostate gland due to the proliferation of smooth muscles following exposure to cardiac glycosides.[72,73] The magnitude of stimulation, which is about 20%–40%, is likely to have clinical implications in some patients with respect to affecting urination. The condition known as Dupuyteren's Contracture is also likely due to the overproduction of fibroblasts induced by low doses of reactive oxygen, with the response following an hormetic dose-response relationship.[74]

A number of immune diseases have also been related to the occurrence of a low dose stimulatory response. While a detailed assessment of hormetic responses of the immune system suggested that most would be beneficial, in about 20% of the cases, the low dose stimulatory response could lead to harmful effects, such as certain autoimmune responses including lupus[75] and tuberculin hypersensitivity.[76]

Hormesis in drug discovery, development and in the clinical trial

Drug discovery, development and clinical trial efficiency could be significantly enhanced if they were guided by principles derived from an understanding of the concept of hormesis. This is the case for drugs designed to kill harmful agents. For example, in screening of agents that may be very effective at killing bacteria, fungi, viruses, yeasts and tumor cells, it would also be important to know whether these agents might be effective stimulating the proliferation of these organisms. It would also be important to know the biological half life of the drug in humans. Ideally, the drug should be effective in killing the harmful agent, have a low capacity to induce cell proliferation at low doses and have a short biological half-life. Nascarella and Calabrese[77] have recently demonstrated that there is an inverse relationship between the capacity to kill yeast cells that are models of human tumor cells and the capacity to induce an hormetic dose response. This makes it even more important to be guided by hormetic principles in the selection of anti-tumor cells. It would be important to know whether this concept could be generalized to the case for harmful bacteria, yeasts and viruses.

The concept of hormesis is central to drug development when the goal of the research is to determine whether the drug can increase human performance (e.g. memory enhancement, bone strengthening). The quantitative features of hormesis will determine the magnitude of the enhanced performance as well as the width of the therapeutic zone. However, it is also doubtful that researchers in these areas are acquainted with the hormesis term, its concept and implications. Of particular concern is how the hormetic concept can guide and affect response expectations, study design and statistical power features of both preclinical studies and clinical trials.

Is the hormetic response more dependent on the organism or the inducing agent?

The question has often been asked as to whether all chemicals can induce hormesis or conversely is the key determinant of the hormetic response the organism. Since all chemicals can induce toxicity, depending on the dose, and hormesis may occur as an overcompensation to a disruption in homeostasis, hormesis would be expected to occur for all agents depending on the experimental context. On the other hand, this is not likely to be the case for agents that induce hormesis via a direct stimulation since these agents are typically going to occur via a receptor-mediated pathway activation process.

Chemical structure and hormesis

The chemical structural determinants of hormesis is a generally unexplored area of investigation. Nonetheless, several groups have reported that structural factors can be determinants of whether an hormetic response will occur or not. This has been intensely studied in the area of anxiotytic drug development. In these investigations, researchers have systematically assessed the presence or absence of an hormetic dose response for each of a large number of highly related chemicals, differing by a single molecular characteristic in a long series of agents. These investigations demonstrated that the hormetic biphasic dose response was reproducibly inducible, but it was highly dependent on certain structural characteristics. These hormetic dose responses have the potential to be predicted via SAR methods.[78-80]

Hormesis and avoiding side effects

Hormesis is a biphasic dose response that often results from the actions of partial agonists and partial antagonists. Part agonists/antagonists are extremely common, being seen in most, if not all, receptor systems. The use of partial agonists/antagonists will diminish the likelihood of adverse effects while creating a broader dose response range over which the response would occur.[78-80] These two features are extremely important for the survival of the individual. One can imagine the survival implications of individuals affected by adverse side effects, ranging from headaches to dizziness, to seeing double, amongst others. A major factor, therefore, in evolutionary success is to minimize undesirable side effects of endogenous agonists. As one can see, with the modern pharmaceutical world, this is not an easy task. However, this could be another critical dimension of hormesis within an evolutionary context.

The hormetic pharmacy

Numerous adaptational-based beneficial responses conform to the hormetic dose response. These responses have the capacity to protect vital organs such as the heart, lungs and brain from a host of damaging stresses/conditions. The hormetic response is also manifested via accelerated healing in various experimental systems.[81] Hormetic responses are also seen with cognitive improvement, in slowing down the onset of various aging processes and in a plethora of neurodegenerative diseases, as well as in reducing susceptibility to a broad spectrum of infectious diseases.[29] Hormesis is also seen in the strengthening of bone, reducing the risks of osteoporosis as well as in treating male sexual dysfunctions and with the capacity to grow hair.[82] Research is now being focused on the next generation of pharmaceuticals called hormetic mimetics. These are endogenous or exogenous agents that activate hormetic adaptively beneficial receptor pathways. It is expected that these agents will be translated into life-enhancing pharmaceuticals.[83] In short, hormetic effects are a central feature of the modern and future pharmacy.

Is science self-correcting and if so, how effective is it?

One of the major revelations of hormetic dose responses is that the scientific community was quick to accept the threshold dose-response model and to incorporate it into the entire spectrum of governmental hazard assessment evaluations and in the risk assessment process. The research and regulatory communities accepted its intellectual framework without validating whether this model could accurately predict responses in the low-dose zone. Since homeopathy and what we now call 'traditional' medicine have been engaged in a bitter conflict for nearly 2 centuries, the hormetic dose-response concept became collateral damage in this social, economic and medical battle.[4] This failure to vet the threshold model was largely a consequence of the conflict between homeopathy and traditional medicine. The field of pharmacology, being an important dimension of traditional medicine, aggressively attacked the writings of Hugo Schulz who had proposed that the hormetic biphasic dose response provided the explanatory principle of homeopathy. Since toxicology emerged from pharmacology, it adopted the dose response perspective of its parent, without much self-initiated investigation. The entire experimental, evaluatory, regulatory and teaching aspects of toxicology came to adopt this 1930s mantra of the dose response. The system surprisingly was never critical of its assumptions about the threshold dose response but always found ways to marginalize the hormesis concept. This is even the situation today, especially as manifested by directions of grant programs that control many professional activities. Furthermore, governmental regulatory agencies continue to find the hormetic dose response extremely challenging and threatening, even though it should help them perform their jobs of serving the public considerably better.

Of particular concern is that the research community, especially in the toxicology domain, can have their intellectual climate directed by regulatory agency toxicology needs. Thus, those persons who control grant funding will largely control the creative directions of the research community. In this way, the non-critical acceptance of the threshold dose-response model has been perpetuated through several generations of pharmacologists and toxicologists, who have simply accepted the assumptions of the handed down threshold dose-response model as being correct. The results of such toxicological intellectual indoctrination have led to the present state of affairs. While progress is being made on changing this perspective, there are also strong governmental institutional controls over how one should think about the dose response and the ability to discuss and assess it openly. This leads back to the question, is science self

correcting? Under normal situation, science is efficiently self-correcting with the best ideas eventually emerging. However, when regulatory agencies control the intellectual agenda and funding, the self-correcting nature of science is undermined as we had seen over the past nearly 80 years when it comes to the critical issue of the dose response.

Discussion

In the late 1980s, there was strong interest in determining whether hormesis was a real biological phenomenon or simply a statistical anomaly. Even the first conference on radiation hormesis in 1985 (see Health Physics, 1987 vol. 52, issue 5 for the peer-reviewed conference proceedings) failed to resolve the issue as reflected in a subsequent debate on the topic in the journal *Science* in 1989 by two of the conference leaders.[84,85] However, the opportunity to more systematically assess the hormetic hypothesis dramatically improved with the creation of the hormesis database,[10,11,13] which has collected and assessed over 8000 examples of dose responses displaying evidence of hormetic dose responses. The database permitted an assessment of questions relating to reproducibility of findings, generalizability across biological models, endpoints and chemical classes, as well as the quantitative features of dose responses and temporal nature of the hormetic response. These initial efforts helped to firmly establish that hormetic responses occurred, were reproducible and not uncommon. Despite this advance, there were other questions, especially those relating to the frequency of hormesis in the toxicological literature and the mechanism or family of mechanisms that could account for hormetic dose responses. With respect to the frequency of hormesis, this was to require the creation of a new hormesis database, one that had a priori entry as well as evaluative criteria. This effort, which involved a separate evaluation of nearly 21,000 articles, revealed the first frequency of hormesis within the toxicological/pharmacological literature, with a value of nearly 40%.[5] Furthermore, there was considerable evidence in the pharmacological literature to account for mechanisms by which direct-acting hormetic dose responses occurred using agonist gradients via receptor subunits to activate stimulatory or inhibitory pathways.[5]

One of the key general observations was that the quantitative features of the hormetic dose response were the same, regardless of the biological system, the endpoint that was measured or the agent that induced it. This was a striking general observation that applied to stimulation of tumor cell proliferation, memory enhancement, immune cell stimulation, plant growth, decreases in anxiety and the broad range of other endpoints reported. These quantitative features of the dose response would occur whether the stimulatory response was of a direct or overcompensatory nature. This suggested strongly that the quantitative features of the hormetic dose response were so widespread and general that it may in fact be a quantitative estimate of biological plasticity independent of species.

While the initial emphasis behind the hormetic reappraisal was environmental risk assessment, the data now indicate that this concept is far more general, impacting any aspect of biology concerned with dose-response relationships. This makes the hormesis concept central to molecular biology as well as pharmacology, toxicology[86] and risk assessment.[87-89]

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