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Hormesis: A Highly Generalizable and Reproducible Phenomenon With Important Implications for Risk Assessment

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From a comprehensive search of the literature, the hormesis phenomenon was found to occur over a wide range of chemicals, taxonomic groups, and endpoints. By use of computer searches and extensive cross-referencing, nearly 3000 potentially relevant articles were identified. Evidence of chemical and radiation hormesis was judged to have occurred in approximately 1000 of these by use of a priori criteria. These criteria included study design features (e.g., number of doses, dose range), dose–response relationship, statistical analysis, and reproducibility of results. Numerous biological endpoints were assessed, with growth responses the most prevalent, followed by metabolic effects, reproductive responses, longevity, and cancer. Hormetic responses were generally observed to be of limited magnitude with an average maximum stimulation of 30 to 60 percent over that of the controls. This maximum usually occurred 4- to 5-fold below the NOAEL for a particular endpoint. The present analysis suggests that hormesis is a reproducible and generalizable biological phenomenon and is a fundamental component of many, if not most, dose–response relationships. The relatively infrequent observation of hormesis in the literature is believed to be due primarily to experimental design considerations, especially with respect to the number and range of doses and endpoint selection. Because of regulatory considerations, most toxicologic studies have been carried out at high doses above the low-dose region where the hormesis phenomenon occurs.

KEY WORDS: Hormesis; U-shaped; adaptive response; low dose; β-curve; stimulation.

1. INTRODUCTION

One of the most fundamental tenets of toxicology is that "the dose determines the poison." This simple phrase provides the basis for the belief that all agents—chemicals and physical phenomena that are capable of producing some effect—have the potential to cause toxicity. Whether toxicity occurs is principally a matter of dose: the greater the exposure to a given agent, the more pronounced or severe the response of a cell or organism. Although this is obvious for well-known poisons such as cyanide, arsenic, lead, and pesticides, it is also true that essential substances such as vitamins, minerals, and even oxygen are toxic at excessive doses.

The tenet that the dose determines the poison provides the basic framework for how toxicologists assess the "hazard potential" of chemical products and materials. The goal of such assessments is to determine the levels of exposure that cause harmful effects, the nature of those effects, and the so-called safe level of exposure. Toxicologic testing is designed,

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therefore, to determine the dose–response relationship. Investigators attempt to describe how a given chemical affects the body at varying doses ranging from very high to very low. The type of information these studies yield will hopefully lead to a determination of the threshold that separates a safe exposure from the early stages of toxicity. Although establishing evidence of a true toxicity threshold is often complicated, the belief is that such thresholds exist for each harmful effect and that they can be determined from toxicology studies.

An offshoot of this belief is the tacit, but pervasive, notion that a chemical is not active in biologically meaningful ways below its toxicity threshold. With this understanding, federal regulatory agencies such as the U.S. Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the Occupational Safety and Health Administration (OSHA) have endeavored to establish acceptable levels of exposure for hundreds of toxic substances. The agencies have operated under the assumption that if the exposure to the agent is below the toxicity threshold, no harm will occur. Moreover, to ensure the likelihood of achieving the goal of a safe environment, the EPA, for example, uses safety or "uncertainty" factors to make sure the potential exposure is even lower than known safe exposure levels. This process results in the creation of exposure standards that are typically far below known toxicity thresholds, as determined by animal studies, and the expected toxicity threshold in humans. In creating such conservative standards, the agencies hope that workers and the general public will not experience any harmful effects from exposure to toxic substances.

1.1. Historical Development of the Hormesis Hypothesis

The assumption that an agent is biologically inactive below the toxicity threshold may not be an accurate description of what actually occurs in cells and whole organisms at subtoxic exposure levels. For example, it has long been known that elements such as minerals and vitamins are toxic at high doses, but essential in lower amounts. Also, pharmaceutical agents such as aspirin have an optimal therapeutic zone: too high a dose causes toxicity, whereas too low a dose renders the drug ineffective.

The hormesis hypothesis states that most, if not all, chemical and physical agents, such as radiation, have the capacity to stimulate biological effects at doses below the toxicity threshold, while causing toxicity at doses above the threshold. Such low-dose stimulatory effects have been referred to as *hormetic* responses from the Greek word meaning "to excite."

The shape of the dose–response curve depicting the maxim "the dose determines the poison" is seen in the high-dose range of the two graphs in Fig. 1.[1] The hormesis hypothesis, that doses below the toxic threshold may be stimulatory, yields a different dose–response relationship. When the response refers to factors such as growth, longevity, fecundity, and weight gain, the curve displays what is called a "β"-curve (i.e., a low-dose stimulation followed by inhibi-

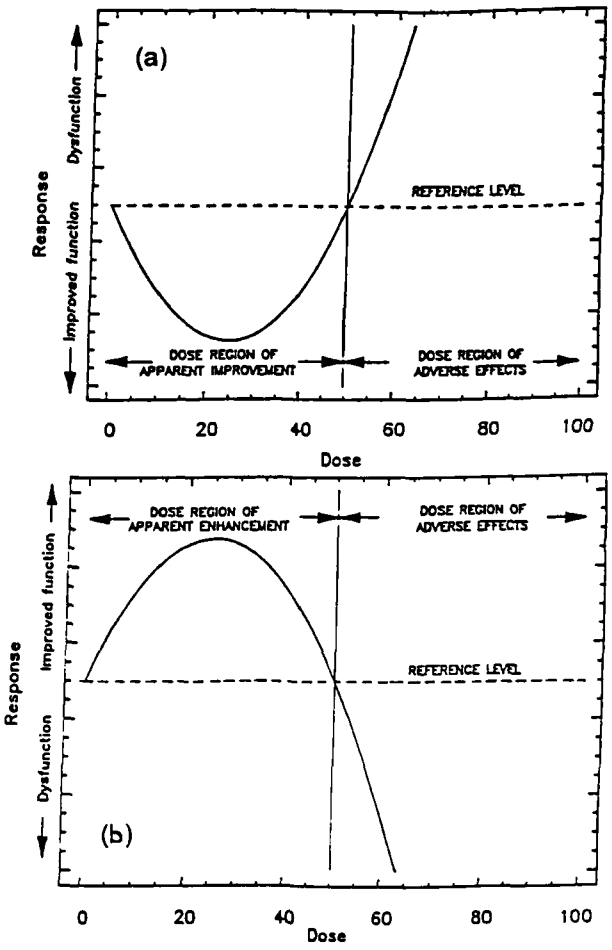

Fig. 1. (*a*) General form of U-shaped dose–response curve showing response relative to a reference level, with a region of apparent improvement (e.g., reduction in dysfunction) as well as a region of toxic or adverse effects. (*b*) Reciprocal of the same curve showing a region of apparent enhancement (e.g., increase above normal level of function) as well as a region of toxic or adverse effects (*Source:* Davis and Svendsgaard.[1])

tion of the stimulated response at higher doses). However, when low doses diminish effects such as mutations, background cancer, or birth-defect incidence, the dose–response curve takes the form of a U- or J-shaped curve. In such cases the low-dose treatment group would display less damage than the unexposed control group. The phenomenon of low-dose stimulatory effects was first reported in 1882 by Rudolph Arndt from experiments with animals, and subsequently in 1888 by Hugo Schulz from studies on yeast fermentation.[2] These findings were integrated with similar observations in what became known as the Arndt–Schulz Law, which stated that all poisons are stimulatory in low doses. This so-called law was believed to be applicable to most, if not all, environmental stressor agents, such as toxic chemicals and radiation, and to most biological systems.

A continuous stream of additional studies on the biological effects of low doses of chemicals and radiation confirmed and greatly extended Schulz's findings. The early confirmation studies, which extended from the 1880s to the 1930s, involved a wide range of biological models including plants, bacteria, and fungi, including yeast. These collective findings represent a substantive body of research that was highly reproducible. The concept of dose–dependent stimulation and inhibition (i.e., hormesis) was not a particularly controversial theory through the early decades of the twentieth century given its widespread observation by highly reputable scientists and the capacity of findings to be independently replicated.[3]

Despite its solid foundation in the scientific literature, the Arndt–Schulz Law attracted hostility and suspicion from its inception, principally because it was used by proponents of homeopathy as an explanatory theory underlying such medical practices. As a result of its close association with homeopathy, and the profound conflict between modern medicine (i.e., allopathy) and homeopathy, the Arndt–Schulz Law came to be seen principally as part of the "belief" system underlying homeopathy rather than as a bona fide biological hypothesis concerning how biological systems adapt to low-dose stressors. This type of guilt by association phenomenon took hold in the 1920s and 1930s such that by the mid-1940s the Arndt–Schulz Law had fallen into disuse. This demise of the Arndt–Schulz Law correlated quite closely with the demise of homeopathy in the United States which had 22 University Homeopathic Medical schools at the turn of the century; only two remained by 1923![4]

Even though homeopathy, and therefore the Arndt–Schulz Law, were not faring well with traditional medical schools, scientists were continuing to observe and report on this phenomenon. In 1943 American scientists at the University of Idaho studying the stimulatory effects of low doses of cedar wood extracts on fungi named the phenomenon hormesis, apparently unaware of the Arndt–Schulz Law.[5] However, the numerous legitimate reports of this phenomenon that continued to accumulate had little impact on how toxicologists designed, conducted, and interpreted their studies.

2. DEVELOPMENT OF THE HORMESIS DATABASE

Over the years, evidence of hormetic responses has been reported in numerous peer-reviewed journals, summarized in books, the subject of national and international conferences, and the object of numerous dissertations. For example, as early as 1924 a journal on cell stimulation was established in Germany (*Zell-Stimulations Forschungen*) to address this phenomenon.[6] Investigators in the area of plant biology published an original research newsletter (*Stimulation Newsletter*) from 1970 to 1981 on the capacity of low doses of radiation to enhance plant growth and yield.[7] More recently, Luckey wrote two books extensively documenting radiation hormesis.[8,9] Independent groups of researchers have organized international scientific conferences in China,[10] Japan,[11] and the United States[12,13] on chemical and radiation hormesis. Of particular importance, with respect to the scientific dimensions of hormesis, are recent advances concerning adaptive-response mechanisms, DNA repair, heat-shock protein induction, acute-phase protein induction, and other responses that have been shown to alter cellular and organismal responses to toxic substances.[14]

Over the past 4 years, we have set forth to assess the viability of hormesis as a scientific hypothesis. We have used as a guide the operational definition that hormesis is characterized by low-dose stimulation and inhibition at higher doses, as seen in the β- and U/J-shaped dose–response curves discussed. A search of various computer databases yielded over 10,000 studies potentially relevant to hormesis. Approximately 3000 of these appeared sufficiently relevant to obtain and carefully assess with objective criteria to screen out those articles that did not present evidence of hormesis, as well as to provide a quantitative ranking of experimental findings with

respect to degree of evidence and documentation for assessment of an hormetic hypothesis.[15,16]

The following criteria were formulated to determine whether a study presented evidence of hormesis: (1) the nature of the study design (e.g., the total number of doses, the number of doses below the toxic threshold, the presence of an adequate control group); (2) the type of endpoints measured; (3) the magnitude and statistical significance of the responses; and (4) the capacity of data replication. These criteria were assigned point values, which were then applied to the studies (Table I). The results provided the basis for determining whether a given study displayed some degree of evidence (i.e., from low to high) of hormesis (Table II). Using this assessment, over 1000 articles reviewed to date have shown evidence of hormesis to some degree. Approximately 10 to 15 percent of the articles display high evidence of hormesis; 25 to 30 percent display a moderate evidence of hormesis.

Table I. Summary of Criteria With Assigned Point Values Used in the Quantitative Evaluation of Hormesis

Study design criteria			
Doses below NOAEL (n)	Point value	NOAEL determined	Point value
1	1	Yes	1
2	2	No	0
3	3	—	—
4	4	—	—
≥5	5	—	—
Response criteria			
Doses statistically significant (n)	Point value	Reproducibility	Point value
1	2	Yes	3
2	4	No	0
3	8	—	—
≥4	16	—	—
Magnitude of response (Percentage control value)			
Inverted U-shaped curve		J-shaped curve	Point value[a]
≥110% ≤ 125%		≤97% ≥ 92%	0.5
>125% ≤ 150%		<92% ≥ 84%	1
>150% ≤ 200%		<84% ≥ 68%	2
>200% ≤ 400%		<68% ≥ 5%	3
>400%		<5%	4

[a] The point value is multiplied by the number of experimental doses falling within the corresponding percentage range. For example, if an experiment has 3 doses exhibiting stimulatory responses within the 125 to 150 percent range, then the total number of points will be 3 × 1.0 = 3.

Table II. Summary of Total Point Ranges for Hormesis Evidence Categories Used in the Quantitative Evaluation of Articles for Evidence of Hormesis

Total point range	Hormesis evidence category
1–2	No–Low
>2–8	Low
>8–12	Low–Moderate
>12–16	Moderate
>16–20	Moderate–High
>20	High

This assessment has revealed much about the types of endpoints (i.e., effects) that display hormesis, the kinds of organisms and agents used in the studies, what may actually be a low dose, the range and magnitude of hormetic responses, and how studies should be designed if the investigator's intent is to study such low-dose phenomena. The findings indicate that low-dose stimulatory responses are not restricted to any particular taxonomic group but are observed broadly across the microbial, plant, and animal kingdoms. This observation, consistent with previous observations,[17,18] is highly significant because it illustrates the broad generalizability of the hormesis phenomenon. Likewise, the types of agents shown to cause hormesis are also without apparent restriction, consisting of agents of seemingly all chemical classes and different types of physical stressors, including various kinds of radiation. The range of biological effects observed with respect to hormesis is also widespread and includes growth, longevity, reproduction, disease incidence, and behavioral aspects. Thus, with respect to generalizability to species, agent, and endpoint, hormesis is potentially far-reaching.

Although the Arndt–Schulz Law was a victim of the conflict between traditional medicine and homeopathy, it is also important to note that its claims were often legitimately criticized as being potentially artifactual resulting from limitations of the study design or normal variation.[19] In fact, most hormetic responses display a maximum stimulatory response less than two-fold greater than controls and are susceptible to being difficult to distinguish from normal variation unless the study is adequately designed and has sufficient statistical power. Likewise, the scientific and public health communities were predominately interested in higher dose phenomena in the 1920s to the 1950s, than is the case today. The issues of assuring the death of harmful bacteria in drinking water, which was critical for community health, as well as

assessing the effects of high concentrations of toxicants to occupationally exposed workers dominated the perspectives of that era.[3,20] Thus, the marginalization of hormesis was the product of a complex series of interacting factors that emerged at approximately the same time, resulting in the diminished role this concept has had on modern toxicologic/risk-assessment thought.

3. EXAMPLES OF HORMETIC RESPONSES

The next section provides a brief summary of experimental findings that provide evidence consistent with the hormetic hypothesis. The entire database of currently approximately 1000 articles is being placed into a relational retrieval-query oriented system with up to 40 fields of information on each experiment and the current evaluation of evidence of hormesis, along with the capacity to alter the evaluation criteria for reevaluation and sensitivity analysis. The following summary provides a limited highlighting of examples in microbial and plant models as well as reproductive and cancer endpoints.

3.1. Microbial Responses

Antibiotics are expected to kill and/or prevent bacteria from reproducing. Studies have shown, however, that low doses of antibiotics such as streptomycin enhance reproduction of certain harmful strains of bacteria at low doses, while killing these strains at higher doses.[21,22] In fact, administering low doses of streptomycin can actually enhance the capacity of the microbe to kill the host. FDA researchers recognized this phenomenon over 50 years ago. It has been observed with penicillin and other antibiotics.[23] As suggested above, the occurrence of hormetic responses in bacteria and other microorganisms is extremely common in the literature. So common in fact are the observations that low doses of toxic substances enhance bacterial growth that this phenomenon became emphasized in multiple well-established microbial textbooks of the 1930s through 1960s,[21,24-26] became a standard experiment in general microbiology laboratories,[21] and the object of questions in comprehensive microbiology standardized testing.[27] Figure 2 represents the entire dose–response relationship typical of numerous antibiotic agents.[28] Note that narrow area of the low end of the dose–response curve is marked stimulation. Although the authors clearly indicated that the entire dose–response spectrum incorporated a low-dose stimulatory range (i.e., the hormetic response), their focus was on the capacity of antibiotics to act in a bactericidal or bacteriostatic (i.e., high-dose) manner.

3.2. Plant Responses

In the area of plant biology, research shows that many agents stimulate growth at low doses, while retarding growth at higher doses. For example, numerous solvents,[29,30] herbicides,[31-34] heavy metals,[35-37] ambient pollutants (e.g., SO_2, NO_2, O_3)[38-41] and ionizing radiation[42-47] have been shown to induce stimulation of root and/or stem growth at low levels. Of particular significance is the substantial body of work dealing with the triazine herbicides, which include the widely used agents atrazine and simazine.[48-56] Low doses of these agents have been repeatedly shown in greenhouse and field studies to enhance growth and yield of various plants in the absence of competing weeds. How these low-level exposures enhance the growth of economically important plant varieties has been a major area of research. As in other areas of hormesis research, the low-dose stimulatory response of plants has been the object of numerous theses/dissertations (see Allender[57] for a recent example).

The hormesis phenomenon has also been commonly observed in the assessment of algal responses to single agents and complex mixtures by numerous investigators including EPA researchers at Gulf Breeze, Florida.[58,59] In fact, the low-dose stimulatory

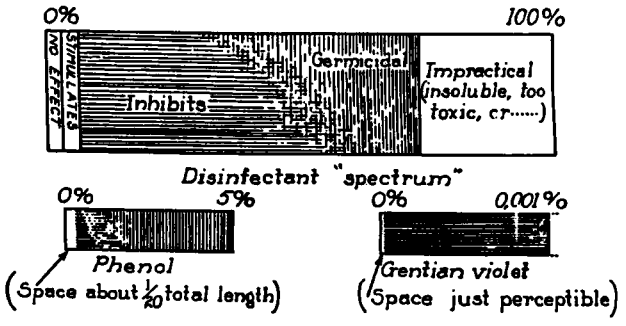

Fig. 2. Disinfectant spectrum. (*Source:* Marshall and Hrenoff.[28])

response became such a predictable feature of their many dose–response relationship investigations with numerous, highly complex effluent mixtures with various algal models that the researchers offered the new term, the SC_{20}, for the concentrations causing 20 percent stimulation of algal growth.[58,59] This term was used in tandem with the more traditional EC_{50} which described the concentration displaying a 50 percent inhibition at the higher end of a concentration spectrum.

3.3. Reproductive Endpoints

In addition, low doses of inorganic substances (such as cadmium, fluoride, and mercury), organic agents (including a wide range of pesticides), and ionizing radiation have been shown to enhance the fecundity of a variety of organisms (Table III), including crustaceans, insects, worms, fish, and mammals. This is particularly relevant to entomologists and others concerned with insect control.[81,82] These findings are also likely to have important implications for the current debate over the dose–response relationship of endocrine modulating agents at low doses. The observation that low levels of environmental toxins can enhance reproductive performance runs counter to the prevailing concept that a reproductive toxin is simply that, and that its capacity to affect the biological system is merely proportional to dose. In these instances, the investigators clearly show the paradoxical influence of dose (i.e., low-dose stimulation) in affecting the final outcome of the study.

4. CANCER AND HORMESIS

Although the above examples of hormetic effects deal with more ecologically oriented endpoints, hormetic responses are commonly reported in mammalian models for the type of endpoints that public health agencies and risk assessment practices are often focused on (e.g., tumor development, DNA damage, teratogenic effects, reduction in longevity). The key feature in assessing the hormetic hypothesis for such human risk assessment endpoints as cancer and teratogenic effects is that the animal model must have a moderately high background (i.e., control) incidence of the endpoint in question. This is because the hormetic hypothesis postulates the occurrence of a J- or U-shaped dose–response relationship for such endpoints. If the control has a negligible background, it will not be possible to observe such a dose–response relationship. Consequently, the evaluation of the hormetic hypothesis for endpoints of the process of carcinogenesis require the selection of models with moderate to high background incidence such as female Sprague-Dawley rats, which display a high background for mammary cancer, the male F344 rat, which displays a high background for testicular cancer, and the Strain A or Swiss mouse, which displays a high pulmonary tumor background incidence. This same strategy also applies to the mutational endpoint as well as tumor promotional endpoints.

With respect to the process of carcinogenesis (i.e., initiation, promotion, and progression), a number of reports have explored broad dose–response relationships and revealed strong consistency with the hormetic hypothesis. Several examples of such studies will be briefly summarized. However, prior to assessing the relationship hormesis to the process of carcinogenesis it must be emphasized that the vast majority (approximately 70 percent to date) of examples of hormesis are found in the ecological toxicology domain where the number of doses employed is often considerably greater than that used in mammalian toxicology studies. Nevertheless, several dozen studies have been published that satisfy both study design and endpoint selection criteria and have been evaluated for evidence consistent with the hormetic hypothesis (Table IV), which enable a preliminary investigation of the generalizability of the hormesis hypothesis for carcinogenesis. Even though the extent of the available data is far less for cancer than ecological toxicity endpoints, a more focused discussion of the cancer data is provided given its significance in modern regulatory risk assessment practices.

4.1. Early Stage (Initiation)

The response (i.e., DNA integrity, poly(ADP)-ribose metabolism, clonogenic survival, and DNA synthesis) of human keratinocytes to low concentrations (6 doses) of the methylating agent *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) over a 500,000-fold concentration range was assessed.[83] At the highest dose DNA unwinding was more extensive than in the untreated controls. However, as the concentration was reduced to a 50- to 0.05-nM range (i.e., the five lowest doses), the DNA unwinding was significantly reduced relative to the untreated con-

Table III. Summary of Organisms Demonstrating Low-Dose Stimulation of Reproduction Organized by Chemical Agent[60]

Agent	Model	Endpoint	Reference
Heavy Metals			
Cadmium	Daphnids	Number of neonates	61, 62
Cadmium	Fathead minnow	Egg production	63
Cadmium	Polychaetes	Fecundity	49
Cadmium	Mice	Number of implants	64
Cadmium	Mice	Number of live fetuses	64
Cadmium	Mice	Maternal weight gain	64
Copper	Polychaetes	Fecundity	49
Copper	Hydra	Bud production	65
Mercury	Polychaetes	Fecundity	49
Zinc	Polychaetes	Fecundity	49
Lead	Polychaetes	Fecundity	49
Lead	Hydra	Bud production	66
Pesticides			
Azinphosmethyl	Aphids	Number of offspring	67–71
Dieldrin	Cotton stainer	Egg production	72
Dieldrin	Mosquito	No. of basal follicles	73
DDT	Mites	Egg production	74
DDT	Mites	Number of offspring	75
DDT	Mites	Number of offspring	76
DDT	Mosquito	No. of basal follicles	73
Carbaryl	Leafworm	Egg production	77
Carbaryl	Rootworm	Egg production	78
Carbufuran	Rootworm	Egg production	78
Dimethoate	Mites	Egg production	79
Sodium flouride	Beetle	Egg production	80
Dicol	Citrus thrips	Number of offspring	81
Esfenvalerate	Citrus thrips	Number of offspring	81
Formetanate	Citrus thrips	Number of offspring	81
Malathion	Citrus thrips	Number of offspring	81
Dinocap	Mice	Maternal weight gain	64
Dinocap	Mice	Number of implants	64
Miscellaneous			
BRDU	Mice	Number of implants	64
BRDU	Mice	Number of live fetuses	64
BRDU	Mice	Maternal weight gain	64
DPH	Mice	Number of implants	64
DPH	Mice	Number of live fetuses	64
DPH	Mice	Maternal weight gain	64
Trypan blue	Mice	Number of implants	64
Trypan blue	Mice	Number of live fetuses	64
Trypan blue	Mice	Maternal weight gain	64

Abbreviations: BRDU = bromodeoxyuridine; DPH = diphenylhydantoin.

trols, with cells at low concentrations having fewer DNA strand breaks than untreated controls. Time course experimentation revealed that at low concentrations the MNNG-induced DNA alterations (e.g., strand breaks) were subsequently repaired in a manner consistent with the overcompensation theory of hormetic effects. The mechanism by which strand breaks were repaired was related to the enhanced activity of poly(ADP)-ribose by the low-dose concentrations of MNNG. Furthermore, colony formation of human keratinocytes in the low-dose range was demonstrated to have a higher survival rate than the untreated controls by nearly 50 percent. The authors concluded with the statement that the "effect of MNNG on human cells at extremely low, but environmentally relevant, doses seems to be quite different from what would be extrapolated from toxicity testing at higher doses."[83]

Table IV. Summary of Data From Cancer Studies Evaluated Using the Quantitative Criteria (see Table I) Listing the Total Scores and Ranking of Evidence of Hormesis (see Table II)

Early stage (initiation)					
Endpoint	Model	Agent	Score	Ranking	Reference
DNA integrity	Human keratinocytes	MNNG	8	Low	83
Poly(ADP)-ribose	Human keratinocytes	MNNG	5	Low	83
Survival	Human keratinocytes	MNNG	4	Low	83
Middle stage (promotion)					
Endpoint	Model	Agent	Score	Ranking	Reference
No. altered hepatic foci	Female rat	Dioxin	9.5	Low–moderate	84
Vol. altered hepatic foci	Female rat	Dioxin	13	Moderate	84
No. altered hepatic foci	Female rat	Phenobarbital	6.5	Low	84
Vol. altered hepatic foci	Female rat	Phenobarbital	10.5	Low–moderate	84
Forestomach cell division	Male rat	Caffeic acid	7	Low	85
Kidney cell division	Male rat	Caffeic acid	4	Low	85
Urinary bladder hyperplasia	Male rat	Saccharin	17	Moderate–high	86, 87
Urinary bladder hyperplasia	Female rat	Saccharin	14	Moderate	86, 87
Late stage (progression)					
Endpoint	Model	Agent	Score	Ranking	Reference
Total tumor no.	Male rat	Saccharin	9	Low–moderate	86, 87
Total tumor no.	Female rat	Saccharin	8	Moderate	86, 87
Pulmonary tumor	Mouse	Methylcholanthrene	14	Moderate	88
Adrenal tumor	Male rat	Dioxin	11	Low–moderate	89
Pancreatic tumor	Male rat	Dioxin	12	Low–moderate	89
Liver tumor	Male rat	Dioxin	13	Moderate	89
Uterine tumor	Female rat	Dioxin	12	Low–moderate	89
Mammary tumor	Female rat	Dioxin	9	Low–moderate	89
Pituitary tumor	Female rat	Dioxin	13	Moderate	89
Liver tumor	Female rat	Dioxin	5	Low	89
Testicular cancer	Male rat	Cadmium	16	Moderate	90

Abbreviation: MNNG = *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

4.2. Middle Stage (Promotion)

The promotion phase of carcinogenesis has also been explored in a limited fashion in the low-dose range, such as caffeic acid–induced cell turnover in the rat forestomach and kidney,[85] the effects of the tumor promoters [i.e., phenobarbital and dioxin (TCDD)] on altered hepatic foci formation in diethylnitrosamine (DEN) pretreated partially hepatectomized rats,[84] and urinary bladder hyperplasia in saccharin-treated rats.[86] These specific cases offer highly suggestive evidence consistent with the hormetic hypothesis. These particular investigations have additional significance because these data have been linked to other research either extending the original observations and/or using the findings to account for what appears to be protective effects on the agent at low doses in cancer bioassays.

4.2.1. Effects of Caffeic Acid on the Stimulation of Cell Division in Forestomach and Kidney of the Male Rat

Caffeic acid (3,4-dihydroxycinnamic acid), a natural phenolic antioxidant that is broadly distributed in vegetables, fruits, and beverages, enhances carcinogenesis via a nongenotoxic process; it does not induce a mutagenic response in bacterial assays. However, it induces forward mutations in cultured mouse lymphoma L5178Y cells and chromosomal aberrations in cultured Chinese hamster ovary cells.[91] Such findings lead the IARC to suggest that caffeic acid might act via a reactive oxygen species or tumor promoting activity, possibly via enhancement of cell division.[92] This hypothesis was subsequently evaluated by Lutz *et al.*[85] in which male F344 rats were fed caffeic acid at five different dietary concentrations

including the controls (i.e., 0, 0.05, 0.14, 0.40, and 1.64 percent) for 4 weeks. The total number of epithelial cells per unit comparison in the forestomach was increased nearly 2.5-fold at the two highest concentrations, whereas no treatment effects were observed at 0.05 percent, the lowest dose studied. However, at the 0.14 percent concentration there was a decrease in these cellular responses by approximately 33 percent. Observations in the kidney closely mirrored those seen in the forestomach with the lowest concentration (0.05 percent) having no treatment effect, the 0.14 percent group responses were about 33 percent that of the controls, and the highest doses were progressively greater than the controls. These U-shaped dose–response relationships in two critical target organs display enhanced cellular division at the higher doses. Lutz *et al.*[85] suggested that the low-dose response of a delayed cell division may account to some extent for the cancer-protective effect seen in previous bioassays as noted above.

4.2.2. Effects of Saccharin on Hyperplasia of the Urinary Bladder

In 1973 the FDA published the results of a multigeneration cancer bioassay concerning the artificial sweetener, sodium saccharin, in which histology was conducted only with F1 generation.[86,87] Of relevance to the concept of tumor promotion was the incidence of urinary bladder hyperplasia data. In this investigation urinary hyperplasia was evaluated over five doses as well as concurrent controls for male and female Sprague-Dawley rats. The data revealed an apparent U-shaped dose response for both sexes for urinary bladder hyperplasia (Table V). Although both sexes displayed the U-shaped dose response, the optimal range of the apparent decrease in the incidence of the hyperplasia is somewhat different, being upshifted in the male. More specifically, the optimal zone (i.e., reduced incidence) for the males was from 0.1 to 1.0 percent of the diet while for the females the optimal zone was from 0.01 to 0.1 percent of the diet. Whether the male would have displayed a further decrease between these two boundary values was not explored. The data for the males were more striking because the background incidence of the urinary hyperplasia are considerably higher and thereby permitted a greater opportunity to assess the possibility of an hormetic response.

Table V. Incidence of Urinary Bladder Hyperplasia for Male and Female Sprague-Dawley Rats in the FDA 1973 Saccharin Study

	Rats with hyperplasia/rats examined, *n* (%)	
Dose (% of diet)	Male	Female
0	10/73 (14)	3/85 (4)
0.01	6/71 (8)	0/81 (0)
0.1	4/81 (5)	0/81 (0)
1.0	4/76 (5)	3/90 (3)
5.0	6/64 (9)	5/88 (6)
7.5	19/62 (31)	0/76 (13)

Source: Downs and Frankowski.[87]

4.3. Late Stage (Progression)

4.3.1. Effects of Saccharin on Bladder Tumor Development

The data on the effects of saccharin on the incidence of urinary bladder hyperplasia (see Table V) suggested that the cancer occurrence may be that of a U-shaped or hormetic dose–response relationship.[86] With respect to the urinary bladder cancer endpoint, the control rats used in the 1973 FDA study had such a low urinary bladder tumor incidence (i.e., 4 percent in males; 0.0 percent in females) that it precluded assessing the hormetic hypothesis for this cancer endpoint.[86]

This hypothesis was subsequently evaluated in the Wisconsin Alumni Research Foundation (WARF) 1974 study, which was similarly designed as the 1973 FDA sodium saccharin bioassay but the number of rats in each treatment group was smaller and the number of treatment groups was reduced from five to three.[81] In this 2-year WARF cancer bioassay, groups of 40 Sprague-Dawley rats (20 male, 20 female) were used, with the sodium saccharin added to the diets at 0, 0.05, 0.5 or 5.0 percent. The overall tumor incidence was increased in the high-dose (5 percent) males compared to controls. However, at the lowest two treatment groups, the females displayed notable decreases in total tumor response (Table VI), a finding consistent with the hormetic hypothesis. The males displayed a similar trend (see Table VI) but because their background total tumor incidence and sample size were low, it was not possible to adequately explore an hormetic hypothesis.

Table VI. Total Number of Tumors for Male and Female Sprague-Dawley Rats in the WARF 1974 Saccharin Study

Dose (% of diet)	Rats with tumors/rats examined, *n* (%)	
	Male	Female
0	3/20 (15)	12/20 (60)
0.05	2/20 (10)	6/20 (30)
0.5	2/20 (10)	9/20 (45)
5.0	14/20 (70)	18/20 (90)

Source: Downs and Frankowski.[87]

4.3.2. Chemically-Induced Pulmonary Tumors

A common cancer bioassay employed in a screening evaluation mode has been the pulmonary tumor incidence in Strain A and Swiss mice. Because these mouse strains display very high spontaneous pulmonary tumor incidence, they offer the opportunity to assess the hormetic hypothesis for this endpoint. To this end, a number of studies have been found in which multiple doses have been employed thus enabling an initial evaluation of the hormetic hypothesis.[88,93,94] These investigations have typically assessed the response to various carcinogenic hydrocarbons. Although these studies tend to vary to some extent with respect to study design, number of doses, dose range, and statistical power, they are remarkably consistant in displaying U-shaped responses. Table VII clearly illustrates the high background pulmonary incidence and the U-shaped nature of the dose–response relationship.[88]

Table VII. Pulmonary Tumors Present at 56 to 79 Weeks of Age in Female Mice Given a Single Subcutaneous Injection of 3-methylcholanthrene as Newborns

3-methylcholanthrene (μg)	Number of mice with tumor/ number of mice	Tumor incidence (%)
0	15/34	44.1
0.005	1/18	5.6
0.015	5/19	26.3
0.046	7/18	38.9
0.137	6/20	30.0
0.400	12/24	50.0
1.200	8/11	72.7
3.700	10/10	100.0
11.100	11/11	100.0

Source: O'Gara *et al.*[88]

4.3.3. Dioxin and Cancer

Evidence to support the hormetic hypothesis includes the results of the 1978 study of Kociba *et al.*[95] which the EPA has relied on for its cancer risk-assessment estimates. The EPA has based its cancer risk assessments on the dose-dependent increase in liver tumor responses at the highest two doses. However, Cook[89] has emphasized that when total tumors are normalized per 100 animals striking U-shaped dose–response relationships became evident for both males and females (Table VIII). The dioxin-treated male rats displayed substantial decreases in tumors of the adrenals, pancreas, and more modestly, the liver. As for the females, the decrease in tumor incidence was principally accounted for by the changes in tumor incidence of the uterus, mammary glands, and pituitary. In fact, even at the lowest dose the females displayed a modest decrease in liver tumors, the critical target organ for the EPA cancer risk assessment.

These findings are consistent with the subsequent work of Pitot *et al.*[84] which displayed a U-shaped dose–response for liver foci formation in partially hepatectomized rats that had received a single initiation dose of DEN followed by graded doses of either phenobarbitol or TCDD. Based on the findings of Kociba *et al.*[95] and Pitot *et al.*[84] Conolly and Anderson[96] developed a biological motivated stochastic initiation promotion model to account for the consistently observed U-shaped responses.

4.3.4. Testicular Cancer

Another cancer bioassay that provided evidence consistent with the hormetic hypothesis was reported by Waalkes *et al.*[90] concerning the effects of cadmium chloride on testicular cancer in Wistar rats. This study involved the administration of six doses of cadmium over a 40-fold dose range (1 to 40 μmol/kg). The control displayed the necessary high background incidence (17.8 percent; 8/45) of testicular cancer. The treatments demonstrated a striking U-shaped dose–response relationship with the lowest dose having only 1 of 30 rats (3.3 percent) displaying a testicular cancer. This study was particularly impressive because it had four doses below the LOAEL thereby making its possible to better explore the nature of the dose–response relationship in the lower dose region (Table IX).

Although the above examples of the carcinogenesis process responses display J- or U-shaped re-

Table VIII. Tumor Frequency in Male and Female Rats Exposed to 2,3,7,8-TCDD

Male rats								
Dose (μg/kg/d)	0		0.001		0.01		0.1	
Rats (*n*)	85		50		50		50	
	No.	Rate	No.	Rate	No.	Rate	No.	Rate
Total tumors	138	162.4	40	80.0	49	98.0	60	120.0
Liver	8	9.4	0	0.0	3	6.0	3	6.0
Pulmonary	2	2.4	0	0.0	0	0.0	2	4.0
Testes	2	2.4	2	4.0	0	0.0	0	0.0
Prostate	0	0.0	1	2.0	0	0.0	0	0.0
Mammary	2	2.4	0	0.0	0	0.0	1	2.0
Pituitary	29	34.1	6	12.0	11	22.0	13	26.0
Pancreas	29	34.1	10	20.0	8	16.0	5	10.0
Adrenal	28	32.9	6	12.0	12	24.0	9	18.0
Female rats								
Dose (μg/kg/d)	0		0.001		0.01		0.1	
Rats (*n*)	86		50		50		49	
	No.	Rate	No.	Rate	No.	Rate	No.	Rate
Total tumors	230	267.4	96	192.0	102	204.0	120	244.9
Liver	9	10.5	3	6.0	20	40.0	34	69.4
Pulmonary	0	0.0	0	0.0	1	2.0	7	14.3
Ovary	3	3.5	1	2.0	1	2.0	0	0.0
Uterus	36	41.9	14	28.0	14	28.0	11	22.4
Cervix/vagina	2	2.3	0	0.0	1	2.0	0	0.0
Mammary	81	94.2	39	78.0	40	80.0	24	49.0
Pituitary	49	57.0	18	36.0	14	28.0	14	28.6
Pancreas	5	5.8	4	8.0	1	2.0	1	2.0
Adrenal	16	18.6	8	16.0	3	6.0	8	16.3

Source: Cook.[89]
Note: Rates are in number of tumors per 100 animals.

sponses, it is possible that an inverted U-shaped response could also occur. For example, if the agent acted principally via an enhancement of cell proliferation, it is possible that a low dose may be promotional whereas higher doses are inhibitory. High-dose indomethacin treatment profoundly prevents DMBA-induced mammary cancer in Sprague-Dawley rats presumably via its inhibitory effects on cell proliferation owing to its inhibition of arachidonic acid metabolism. However, Tripathi et al[97] reported that the response to indomethacin is dose-dependent with the cell proliferation, as measured by thymidine uptake, enhanced by 30 to 40 percent over 10^{-10} to 10^{-6} M, while inhibitory at higher concentrations (10^{-5} to 10^{-4} M). The implications of these findings suggest that the tumor suppressive effects of indomethacin seen at high levels may be reversed at low levels. This biphasic response reflects the fundamental tenets of the hormesis hypothesis (i.e., the biphasic nature of the dose-response).

Table IX. Incidence of Testicular Tumors in Wistar Rats Treated With Cadmium Chloride

Dose (μmol/kg)	Rats (*n*)	No. of tumors (%)
0	45	8 (17.8)
1.0	30	1 (3.3)
2.5	29	3 (10.3)
5.0	30	3 (10.0)
10.0	30	4 (13.3)
20.0	29	21 (72.4)
40.0	29	24 (82.8)

Source: Waalkes *et al.*[90]

5. HUMAN RESPONSES

A well-studied and accepted example of the J-shaped curve in humans is the relationship between ethanol consumption and the risk of cardiovascular disease. Numerous reports from the epidemiologic literature indicate that individuals who consume sev-

eral alcoholic drinks per day have a demonstrably lower risk of heart attack than those who consume excessive amounts of alcohol and those who abstain from drinking alcohol.[98-101] Of importance is that the response seems to have a reasonably solid underlying mechanistic explanation involving the enhancement of the HDL proteins—the so-called good cholesterol—which are known to protect against cardiovascular disease.[102-105]

6. HORMETIC MECHANISMS

When the imposition of an external stressor agent (e.g., pollutant exposure) challenges the adaptive capacity of a biological system, the system typically more than compensates for the initial disruption and/or damage, leading to the net stimulatory (i.e., hormetic) response.[17] Thus, hormesis represents an overcompensation to an alteration in homeostasis. When the dose progressively increases, the system's capacity to compensate becomes overwhelmed, the "no observed adverse effects level" (i.e., toxicity threshold) is exceeded, and evidence of toxic effects becomes manifest via biochemical and histologic endpoints.

The range of hormetic effects, such as increased growth, fecundity, longevity, and decreased disease incidence, suggests the involvement of thousands of genes thereby implying that hormetic mechanisms affect basic biological processes. Nonetheless, investigators often direct their attention to mechanisms closely attuned to aspects through which biological protection may be mediated. For example, there is substantial evidence that very specific alterations in patterns of gene expression in numerous species occur in response to toxicant exposures. Such responses fall into one or two classes: (1) those resulting in an enhanced metabolic capacity for detoxification of the particular toxicant; or (2) those that offer more general protection against cellular damage caused by a wide variety of agents.[14,106]

The field of molecular biology has recently provided tools to enhance understanding of the mechanistic foundations of hormetic dose–response relationships. Of particular interest is the "adaptive response" phenomenon that occurs in both radiation and chemical toxicity.[107,108] In general, if a low—and often nontoxic—exposure to radiation or a toxic chemical is administered either to cells in culture or to whole organisms, and is then followed by a massive exposure to the agent that would normally seriously injure or even kill the cells or organisms, the preexposed cells or organisms display remarkable protection from toxicity and lethality. This phenomenon has been intensely studied for over a decade, resulting in hundreds of research papers providing important insights into how the protection occurs, the nature of the dosing that elicits the response, why some cells and organisms differ in their responsiveness, and the overall generalizability of the phenomenon. Although the adaptive-response phenomenon technically differs from hormesis as described here, the induction of molecular adaptive responses at the low levels used in these experiments may provide a sound model for how hormetic processes are triggered.

7. TEMPORAL FACTORS IN ASSESSING HORMESIS

The assessment of the hormetic response may be profoundly enhanced by the incorporation of a temporal component within the study design. Such incorporation of a repeat measures design feature enables an evaluation of the underlying dynamic features of the process of hormesis. Consideration of temporal features within the study design was the crucial factor leading Stebbing[17] to define hormesis as an overcompensation to an initial disruption in homeostasis. More specifically, he observed an initial dose-dependent toxicity followed by a limited overcompensation response. The net result after the overcompensation was the widely observed β-curve. Recognition of the temporal dimension in the hormetic response becomes a critical feature affecting study design and endpoint selection. Although Stebbing has provided a modeling feedback system approach to account for the dynamics of hormetic responses,[17] the recognition that hormesis resulted from an overcompensation to a disruption in homeostasis can be traced back to Townsend,[109] Hofmann,[110] Branham,[111] Smith,[112] and others. Figure 3 illustrates the temporal influence on the development of the β-curve, showing dose-dependent inhibitory responses soon after exposure followed by the modest overcompensation response (i.e., hormesis).

The recognition of such an overcompensation response as being fundamental to hormesis provides a basis for understanding why the typical stimulatory response is usually modest, why the hormetic response may be viewed in reference to the traditional NOAEL, and why the size of the response is over a

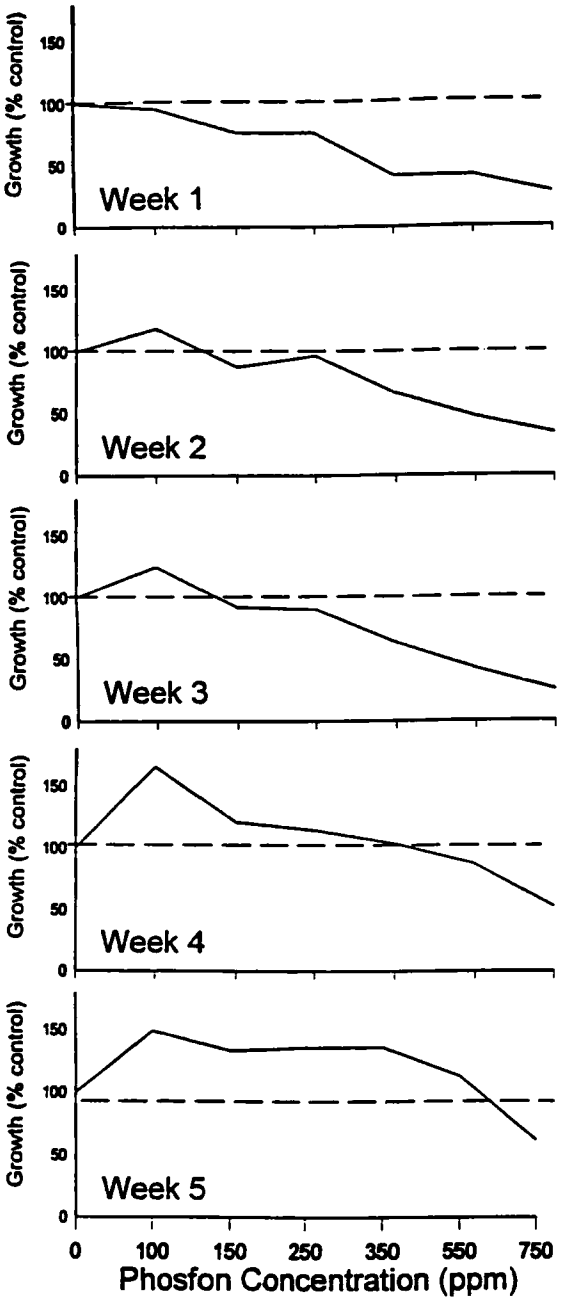

Fig. 3. Dose–response curves at various time points during an experiment with peppermint plants exposed to various concentrations of phosfon (Data from Calabrese.[113])

limited range (up to 20-fold). Because the hormetic effect is a response to an initial low to modest disruption in homeostasis, there is no need to grossly overcompensate, but simply to assure that equilibrium will be reached. This leads to the modest 30 to 60 percent greater than control response. The fact that hormesis is seen in reference to the NOAEL is likewise in part because it is a phenomenon that is intimately related to the concept of toxic threshold. Thus, the dose–response characteristics seen with hormesis are consistent with a systems-based feedback response that is in turn linked to an evolutionary-based adaptive response to low-level environmental stressors.

8. DISCUSSION

8.1. Why Does Hormesis Continue to Be Ignored?

Despite the substantial body of evidence, why does hormesis remain a stumbling block for many scientists? First, although there are many examples of hormetic dose–response relationships, such studies actually constitute a very small percentage of the whole toxicologic database. For example, about 500,000 animal toxicology studies have been published in the peer-reviewed literature since 1900.[15] Yet, in order to have a reasonable chance of obtaining a high score for evidence of hormesis based on our evaluation system, a study often requires at least six total doses, with at least three doses within a factor of 10 below the NOAEL. Studies so designed are relatively few (based on the single-exposure carcinogen database): only about 1 out of every 500 published studies would satisfy the dose conditions.[114] This alone is sufficient to explain why many toxicologists know little about the concept of hormesis.

Second, as seen in Fig. 4, hormetic responses have been repeatedly shown to occur over a limited dosage range and response magnitude. For example, the average maximum stimulatory response is about 30 to 60 percent greater than the control. Examples where the increase is several fold greater than the control are atypical. When responses are seen in the percentage increase zone rather than in the fold increase zone, the stimulatory response may often be interpreted as normal variability rather than a real stimulation (i.e., a "false-positive" response) and reproducibility becomes an important issue. Recognition that hormetic responses are of a generally modest nature places greater experimental design demands on the researchers; more treatment groups are needed to define the nature of the dose–response relationship, especially in the low-dose zone where

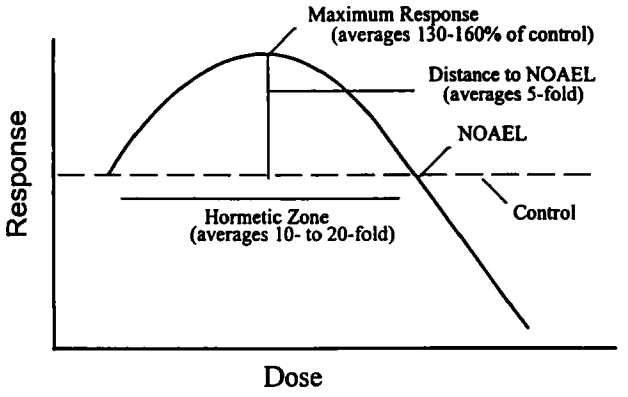

Fig. 4. Dose–response curve depicting characteristics of the chemical hormesis zone. Note that the magnitude of stimulation is typically 30 to 60 percent greater than control values while the zone of stimulation extends on average approximately over a 10-fold range. (*Source:* Calabrese and Baldwin.[115])

the hormetic response would be expected to occur. In addition, the more limited nature of the response requires that greater attention be devoted to sample size and statistical power issues. Such constraints suggest that in experiments designed to investigate the low-dose area, it is prudent to use more subjects in the low-dose treatment groups than at the higher doses, owing to the treatment effects' limited magnitude and the variability of response.

8.2. Why Is Hormesis Not Seen More Frequently?

Although the hormetic response appears to be highly generalizable, numerous examples exist where hormesis is not observed even though studies have been seemingly adequately designed to detect hormetic responses. According to Stebbing, the principal factor affecting the observance of hormesis is the presence of an initial inhibitory (or toxic) challenge capable of inducing a counter-response (or overcorrection).[18] If the inhibitory challenge is too intense, then the overcorrection will be abolished. Likewise, if the challenge is too weak, then the overcorrection may not be induced or may be too small to discern. Assuming that the overcorrection is induced, then four factors have been identified as contributing to the absence of a convincing hormetic response. In general, hormesis is not observed when (1) study design parameters preclude its detection, (2) environmental (or culture) conditions do not favor its expression, (3) complexity of the test model masks its detection, and/or (4) negligible background values preclude its detection. These factors, acting alone or in combination, may affect a study's ability to express an hormetic response and are discussed below.

8.2.1. Study Design Parameters Preclude Its Detection

With few exceptions, the characteristics of the dose–response curve (β-curve) defining a hormetic response are very specific and confined to a relatively small concentration range.[115] Selection of an appropriate number and range of doses that will produce not only a NOAEL, but also clearly define the shape of the low-dose stimulatory response is a crucial study design requirement for the detection of hormesis. Because most hazard assessment oriented toxicology studies focus on establishing NOAELs, LOAELs, and FELs, their study designs may not include doses within a potential hormetic range (i.e., intentionally below NOAEL doses), and therefore commonly fail to produce evidence or data for hormetic responses. For example, Fiskesjo, who utilized the 96-hour Allium test (assessment of onion root growth following 96-hour exposure to test agents) to determine the relative toxicity of metal ions based on EC_{50} values observed only growth inhibition even at the lowest mercury concentrations tested (i.e., 0.2 mg/L $HgCl_2$ and 0.01 mg/L CH_3HgCl).[116] Subhadra *et al.* however, using the same protocol and model but a dose range encompassing lower concentrations, reported growth stimulation at concentrations of 0.01 and 0.001 mg/L $HgCl_2$ (104 and 108 percent of control, respectively) and at 0.0001 mg/L CH_3HgCl (113 percent of control).[117]

Duration of exposure is another essential parameter which, in contrast to the well-defined characteristics of the β-curve, cannot be generalized. The optimum duration of exposure for observation of hormetic dose-responses depends on model, endpoint, and agent. The hormetic response may become more pronounced with time as seen in egg production of female microarthropods exposed to low concentrations of dimethoate.[79] On the other hand, hormesis may be a transient event as observed in the initial increase in growth rate of hydroid colonies exposed to low concentrations of copper.[18] Other investigators (e.g., Vichi and Tritton[118]) report hormetic results that are not strongly time dependent. Unless a study design incorporates not only an appropriate duration of exposure, but also adequate selection of

time points for data collection, the occurrence of a hormetic response may be missed.

8.2.2. *Environmental (or Culture) Conditions Do Not Favor Its Expression*

Conditions favorable for hormetic expression are best shown in studies comparing data from experiments conducted under different experimental conditions. The results of these studies suggest that suboptimal conditions may enhance the hormetic response in a wide range of experimental models and agents.

In plants, data show that increased growth and nitrogen content of corn,[50,119] rye,[51] and peach and apple trees[120] treated with subtoxic levels of simazine are greatest when nitrate and temperature are at suboptimal levels. Likewise, pronounced stimulation of growth of lettuce plants exposed to low dose H_2S was observed during periods of cooler, rather than comparatively higher, greenhouse temperatures.[121]

At suboptimal salinity, enhanced survival was observed in zoeal mud crabs exposed to mid-range concentrations of jet fuel water-soluble fractions compared to optimal concentrations.[122] This response was not observed at higher salinities where survival was high at all concentrations. In other crustaceans, low-dose copper stimulation of instar growth was observed in cladoceran species maintained at a low, suboptimum food level, but not at the high food level.[123]

In experiments with human and murine cells, Vichi and Tritton were able to demonstrate significant low-dose adriamycin-induced growth stimulation only in cultures containing high-density cells in partially exhausted medium.[118] The authors concluded that in order to observe growth stimulation with an otherwise cytotoxic chemical one necessary requirement is that the medium must be incomplete (i.e., depleted of growth-promoting components and/or containing suboptimal serum concentrations). The authors speculated that in vivo environments would also need to be nutritionally suboptimal to obtain stimulation.

Most studies are believed to be conducted under apparent optimal conditions to ensure that the health of the organism is not compromised. If suboptimal conditions enhance the expression of hormesis, then the capacity of a study to detect low-dose stimulation may be reduced under optimal conditions.

8.2.3. *Complexity of the Test Model Masks Its Detection*

Stebbing and Heath have proposed that a hierarchical scheme of homeostatic control subsystems operate at different levels of cellular organization.[124] According to this hypothesis, homeostatic disturbances are progressively dissipated as they cascade through an organism's hierarchical system. In theory, more complex organisms (i.e., those with more levels of organization), would have a greater capacity to counter exogenous disturbances. It follows that this greater capacity would increase the probability that the response of lower level subsystems will be obscured. Therefore, studies utilizing complex, whole animals as test models may not be able to detect an hormetic response occurring at a subsystem. More primitive in vivo models or in vitro cell or organ models may more efficient experimental systems for observing a subsystem stimulation.

Cassida and Allen, in developing a screening methodology for evaluating the effect of insecticides on plants, found that in order to detect a difference between toxicity and stimulation an in vitro system utilizing small sections of seedling bean stems was necessary to produce consistent, easily interpretable results.[125] The authors report problems encountered with more complex models such as inhibition of root growth by application of inhibitory as well as stimulatory (e.g., auxins) substances to aerial parts of the plant.

8.2.4. *Negligible Background Values Preclude Its Detection*

As discussed in the section on Cancer and Hormesis, the experimental model's background incidence of certain endpoints (e.g., tumor incidence, DNA damage) determines the study's capability to detect hormetic responses. If the control displays a negligible background response, then it will not be possible to observe a J- or U-shaped dose–response relationship.

8.3. Potential Significance of Hormesis to Environmental and Health-Related Decision-Making

What is the potential significance of hormesis, especially to federal regulators and environmental

health scientists? Although it is too early to say precisely, the possibilities are intriguing.

Federal regulatory agencies, employing the concept of threshold responses, have adopted risk-assessment procedures that assume noncarcinogenic toxic substances cause harmful effects above a toxic threshold, below which no adverse effects are expected to occur. Although the agencies have focused on adverse effects, they have given virtually no consideration to whether beneficial effects might occur below the threshold except in the case of essential agents such as selenium, copper, and so on.[126] However, the generalizability of hormesis to most compounds suggests that agencies such as the EPA, FDA, and OSHA should carefully consider applying this concept to their risk assessment procedures.

Each agency might have a different perspective on evaluating the role of hormesis based on its respective responsibilities. For example, OSHA's occupational health standards are designed to permit higher exposure levels than community exposure standards. The EPA's and the FDA's risk-assessment procedures are currently designed to protect the public against the harmful effects of toxic substances. In fact, the resultant standards are believed to be sufficiently conservative so as not only to achieve the protection goal, but also to reduce the exposure below the estimated hormetic dose.[126] If the agent produces a significant beneficial effect (such as enhanced longevity) at low doses, and such low-level exposures are not permitted, these conservative standards may, in fact, be more harmful than less stringent standards.

Thus, all regulatory decisions on noncarcinogenic chemical and physical agents should, at a minimum, address how the proposed standard affects possible hormetic responses. Every exposure standard should provide a description of the biological and population-based responses for the entire dose–response relationship, not just for the higher doses, which has been the standard practice to date. Regulatory agencies need to provide information on the complete set of public health implications regarding each exposure standard beyond the benefit of avoiding potentially harmful exposures. Moreover, if the standard, by preventing low-level exposure to a regulated agent, has eliminated the attainment of potentially beneficial effects, agencies need to recognize and justify this result to the public in future standard-setting activities.

Several reports have been published concerning the incorporation of the concept of hormesis in the RfD process.[127-129] These efforts offer specific approaches by which the current RfD concept can be expanded to not only prevent adverse effects with an adequate margin of safety but to ensure that possible beneficial effects are not lost. This latter point needs to be incorporated into a broader and more expanded concept of the RfD.

Risk assessment regarding exposure to carcinogenic agents could also explicitly address possible hormetic responses. With some exceptions, federal agencies currently assume that there is no safe level of exposure to carcinogens. However, the hormesis concept suggests not only that this is incorrect, but that low levels of exposure to many carcinogens may actually have some net benefit to the organism.

It is recognized that how the concept of hormesis relates to the process of carcinogenesis is likely to be complex, having the capacity to inhibit, and under certain conditions possibly enhance, the process of carcinogenesis in the low-dose zone depending on the mechanisms involved and the collective summation or result of multiple hormetic mechanism inductions. Although the clarification of such interactions will remain to be developed for particular cases, there is little question that the underlying assumptions of the cancer risk dose–response relationship need to incorporate an hormetic component into its framework.

The implications of the hormesis concept for cancer risk assessment is that it provides a biologically based foundation supporting the concept of thresholds for many, if not most, carcinogenic responses. It also suggests that the regulatory "goal" of exposure to carcinogenic agents should not be zero as has been the case (e.g., carcinogens in drinking water[130]), but a "goal" aimed at achieving an optimized health-based response. While these efforts will require considerable future development it is clear that the concept of hormesis has the potential to enhance current approaches for risk assessment for both noncarcinogens and carcinogens.

9. SUMMARY

This present paper summarizes an extensive ongoing effort to evaluate with objective methods (e.g., processes with negligible inter- and intra-evaluator variability) the consistency of experimental findings with hormesis as a biological hypothesis. In fact, we believe that the establishment of the current general evaluation protocol offers the means to enhance a more objective assessment of the hormetic hypothesis

and provides an important advance in moving hormesis from an ideology back to an hypothesis. Although we believe that the criteria selected and their subjective weightings are credible means for assessing experimental data, the current system would be open to modifying both qualitative and quantitative weighting criteria. Preliminary efforts with alternative criteria strongly suggest that the general conclusions as to the robustness and generalizability of the hormesis hypothesis are not likely to be significantly modified regardless of reasonable changes in evaluation criteria.

Perhaps the most unexpected finding of the effort to assess hormesis as a biological hypothesis has been the extraordinary number of high-quality reports in the literature that have observed this phenomenon. Recognition of the hundreds of studies displaying evidence of hormesis offers a remarkably strong foundation to provide objective quantitative analysis of this phenomenon. Not to be ignored is that the criteria adopted to assess hormesis were deliberately selected to be rigorous, thereby excluding large numbers of studies from achieving moderate or higher evidence rankings even though many appear to provide bona fide evidence of hormesis. In fact, in the extensive section of this paper on hormesis and carcinogenesis only one paper was found to have more than moderate evidence of hormesis (see Table IV). As mentioned earlier, unless the study has sufficient doses below the NOAEL which display statistically significant responses, it is practically impossible to achieve a classification of more than low-to-moderate evidence of hormesis in the current system.

It should be noted that the criteria were designed to assess experimental findings for possible hormetic responses. Consequently, these criteria are presently unable to be readily applied to epidemiologic data. Therefore, epidemiologic studies of alcohol[97-101] and radon,[131,132] which are purported to show possible hormetic effects, are not addressed in the present methodology.

Within this evaluative context, we now have approximately 1000 primary research papers in the peer-reviewed literature, with an average of three endpoints per study, for which there is evidence (i.e., low to high) consistent with the hormetic hypothesis. This extensive effort was undertaken to establish (1) some measure of the occurrence of hormesis in the literature, and (2) the characteristics of the phenomenon itself (e.g., magnitude and range of the response, relationship to the NOAEL, types of endpoints, biological model employed, etc.). Prior to this effort no such extensive documentation and evaluation has been undertaken.

Other attempts have been undertaken to assess the probability of finding hormetic effects in the toxicological literature. For example, Davis and Svendsgaard[1] undertook a randomized search of a substantial number of leading journals over a selected number of years to attempt to determine the frequency with which hormesis may be seen in the literature. This approach used different criteria than the present study for attributing evidence of hormesis from the present study. They confirmed support for hormesis if a treatment deviated from the control by 5 percent, a phenomenon observed in 12 percent of the experimental studies.

Although it has been argued here that hormesis represents an overcompensation to a disruption in homeostasis, our investigation of U-shaped dose-responses, with specific temporal and dose–response characteristics, has revealed the existence of a number of well-established exceptions to this generalized phenomenon.[115] For example, apparent hormetic responses exist without a prior inhibitory response, with the stimulatory range over several orders of magnitude, and with the stimulatory response in a 5- to 10-fold range.[115] Such evidence suggests that there is a broad family of U-shaped dose–response relationships with hormetic responses (i.e., overcompensation to a disruption in homeostasis) being one type of such low-dose stimulatory dose–response relationships. Although U-shaped responses, which derive from an apparent overcompensation to a disruption in homeostasis, are argued as being hormetic, the number of studies involving temporal features, which establish support for the hormesis theory of Stebbing,[17] are limited to less than 5 percent of the total database. In contrast, over 90 percent of the data seem to conform to the broad features of the so-called hormetic dose-response; that is, a modest stimulatory range of 10- to 20-fold, a maximum stimulatory response of 30 to 60 percent over controls, and a maximum response being within a factor of 4 to 5 of the NOAEL. Because most dose-responses in the database resemble those experiments that have evidence of a temporally based overcompensation response, it has been assumed that they involved a similar disruption in homeostasis and a limited overcompensation (or hormetic) response. Although this appears to be a logical conclusion there are insufficient data to prove this position. Thus, although all U-shaped dose-responses are often called hormetic, this is most likely a convenient overgeneralization

that has been serving a functional role but one that will be markedly refined in the future.[115] The recognition that there may be a broad family of mechanism-based U-shaped dose–response relationships is an important interpretation with potentially highly significant implications for risk assessment.

Much remains to be explored within the realm of hormetic effects, but it is hoped that the current efforts may stimulate the broader scientific community to explore via experimental and assessment means hormesis as a viable hypothesis with potential important implications for society. Although hormesis has not received the attention that it warrants, it is hoped that this broad biological phenomenon may become a legitimate area of inquiry and not be forced to be rediscovered as a "novel" hypothesis by a new generation of toxicologists in the twenty-first century.

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REFERENCES

1. J. M. Davis, and D. J. Svendsgaard, "U-shaped dose-response curves: Their occurrence and implications for risk assessment," *BELLE Newslett.* **1,** 4–15 (1992).
2. H. Schultz, "Ueber Hefegifts," *Pflugers Arch. Ges. Physiol.* **42,** 517–541(1888).
3. E. J. Calabrese, and L. A. Baldwin, "Chemical hormesis: Its historical foundations as a biological hypothesis," *Toxicol. Pathol.* **27,** 195–216 (1999).
4. D. Ullman, *Discovering Homeopathy* (North Atlantic Books, Berkeley, CA, 1991).
5. C. M. Southam, and J. Ehrlich, "Effects of extract of Western Red-Cedar Heartwood on certain wood-decaying fungi in culture," *Phytopathol.* **33,** 517–524 (1943).
6. *Zell Stimulations Forschungen,* M. Popoff and W. Gleisberg (eds.) (Paul Parey, Berlin, 1924–1930).
7. *Stimulation Newsletter,* (Institut fur Strahlenbotanik der Gesellschaft fur Strahlen- und Umweltforschung, Munich, 1970–1981).
8. T. D. Luckey, *Hormesis with Ionizing Radiation* (CRC Press, Boca Raton, FL, 1980).
9. T. D. Luckey, *Radiation Hormesis* (CRC Press, Boca Raton, FL, 1991).
10. S. Z. Liu (ed.), *Proceedings of the International Symposium on Biological Effects of Low Level Exposures to Radiation and Related Agents. Changchun, China.* (Princeton Scientific Publishers, Princeton, NJ, 1994).
11. T. Sugahara, L. A. Sagan, and T. Aoyama (eds.), *Low-Dose Irradiation and Biological Defense Mechanisms* (Elsevier Science Publishers, Amsterdam, 1995).
12. E. J. Calabrese (ed.), *Proceedings of the Second BELLE Conference on Dose-Response Relationships,* (Lewis Publishers, Boca Raton, FL, 1994).
13. E. J. Calabrese (ed.), "Proceedings of the Third BELLE Conference on Toxicological Defense Mechanisms and the Shape of the Dose-Response Relationship," *Environ. Health Perspect.* **106** (suppl 1), 275–394 (1998).
14. R. W. Hart, and L. T. Frame, "Toxicological Defense Mechanisms and How They May Affect the Nature of the Dose-Response Relationships," *BELLE Newslett.* **5,** 1–16 (1996).
15. E. J. Calabrese, and L. A. Baldwin, "The dose determines the stimulation (and poison): Development of a chemical hormesis database," *Int. J. Toxicol.* **16,** 545–559 (1997).
16. E. J. Calabrese, and L. A. Baldwin, "A quantitatively-based methodology for the evaluation of chemical hormesis," *Human Ecol. Risk Assess.* **3,** 545–554 (1997).
17. A. R. D. Stebbing, "A theory for growth hormesis," *BELLE Newslett.* **6,** 1–11 (1997).
18. A. R. D. Stebbing, "Hormesis—Stimulation of colony growth in *Campanularia flexuosa* by copper, cadmium, and other toxicants," *Aquat. Toxicol.* **1,** 227–238 (1981).
19. A. J. Clark, *Handbook of Pharmacology* (Verlag Von Julius Springer, Berlin, 1937).
20. E. J. Calabrese, and L. A. Baldwin, "The Marginalization of Hormesis," *Toxicol. Pathol.* **27,** 187–194 (1999).
21. A. J. Salle, *Fundamental Principles of Bacteriology* (McGraw-Hill, New York, 1939).
22. C. Lamanna, "Growth stimulation by sulfanilamide in low concentration," *Science* **95,** 304–335 (1942).
23. W. S. Miller, C. A. Green, and H. Kitchen, "Biphasic action of penicillin and other similar sulphonamides," *Nature* **155,** 210–222 (1945).
24. C. E. Clifton, *Introduction to Bacterial Physiology* (McGraw-Hill, New York, 1957).
25. C. Lamanna, and M. F. Mallette, *Basic Bacteriology: Its Biological and Chemical Background. Third Edition,* (Williams & Wilkins, Baltimore, MD, 1965).
26. K. Thimann, *The Life of Bacteria,* 2nd ed. (Macmillan, New York, 1963).
27. C. W. Kim, *Microbiology Review* (Medical Examination Publishing Company, Flushing, New York, 1968).
28. M. S. Marshall, and A. K. Hrenoff, "Bacteriostasis," *J. Infect. Disease* **61,** 42–54 (1937).
29. D. J. Morre, B. J. Rogers, and R. Gamble, "Promotion of plant growth by long chain alcohols and organic solvents," *Phyton* **22,** 7–12 (1965).
30. D. J. Morre, "A protein disulfide-thiol interchange protein with NADH: Protein disulfide reductase (NADH oxidase) activity as a molecular target for low levels of exposure to organic solvents in plant growth," *BELLE Newslett.* **6,** 25–33 (1998).
31. W. J. Allender, "Effect of trifluoperazine and verapamil on herbicide stimulated growth of cotton," *J. Plant Nutr.* **20,** 69–80 (1997).
32. W. J. Allender, G. C. Cresswell, J. Kaldor, and I. R. Kennedy, "Effect of lithium and lanthanum on herbicide induced hormesis in hydroponically-grown cotton and corn," *J. Plant Nutr.* **20,** 81–95 (1997).
33. E. J. Calabrese, and K. J. Howe, "The effects of phosfon on the growth of *Mentha piperita* L.," *Physiol. Plantar.* **37,** 163–165 (1976).
34. S. J. Wedman, and A. P. Appleby, "Plant growth stimulation

by sublethal concentrations of herbicides," *Weed Res.* **12,** 65–74 (1972).
35. H. Brown, and M. H. Martin, "Pretreatment effects of cadmium on the root growth of *Holcus lanatus* L.," *New Phytol.* **89,** 621–629 (1981).
36. H. Hirt, G. Casari, and A. Barta, "Cadmium-enhanced gene expression in suspension-culture cells of tobacco," *Planta* **179,** 414–420 (1989).
37. J. A. C. Verkleij, P. Koevoets, J. Van, T. Riet, T. Bank, Y. Nijdam, and W. H. O. Ernst, "Poly(y-glutamylcysteinyl) glycines or phytochelatins and their role in cadmium tolerance of *Silene vulgaris,*" *Plant. Cell Environ.* **13,** 913–921 (1990).
38. P. I. Coyne, and G. E. Bingham, "Photosynthesis and stomatal light responses in snap beans exposed to hydrogen sulfide and ozone," *J. Air Pollut. Cont. Assoc.* **28,** 1119–1123 (1978).
39. A. G. Chakrabarti, "Effects of air pollutants on seed germinability," *Bull. Environ. Contam. Toxicol.* **21,** 749–755 (1979).
40. J. P. Bennet, K. Barnes, and J. H. Shinn, "Interactive effects of H_2S and O_3 on the yield of snap beans (*Phaseolus vulgaris* L.)," *Environ. Exper. Bot.* **20,** 107–114 (1980).
41. D. Eamus, J. D. Barnes, L. Mortensen, H. Ro-Poulsen, and A. W. Davison, "Persistent stimulation of CO_2 assimilation and stomatal conductance by summer ozone fumigation in Norway spruce," *Environ. Pollut.* **63,** 365–379 (1990).
42. K. Sax, "The effect of ionizing radiation on plant growth," *Am. J. Bot.* **42,** 360–364 (1955).
43. K. Sax, "The stimulation of plant growth by ionizing radiation," *Rad. Bot.* **3,** 179–183. (1963).
44. A. H. Sparrow, and J. E. Gunckel, "The effects on plants of chronic exposure to gamma radiation from radiocobalt," in *Proceedings of the International Conference on the Peaceful Uses of Atomic Energy, Volume 12, Geneva Switzerland* (United Nations, New York, 1956) pp. 52–59.
45. N. V. Timofeev-Resovskil, and N. V. Luchnik, "Stimulation of plants by radiation and its possible theoretical interpretation," in *Radiobiology* (Consultants Bureau, Inc., New York, 1959).
46. L. B. Breslavets, in A. H. Sparrow, ed., *Plants and X Rays.* Translated by A. Elbl. (The American Institute of Biological Sciences, Washington, DC, 1960).
47. M. M. Miller, and W. M. Miller, "Radiation hormesis in plants," *Health Physics,* **52,** 607–616 (1987).
48. J. R. Freney, "Increased growth and uptake of nutrients by corn plants treated with low levels of simazine," *Aust. J. Agric. Res.* **16,** 257–263 (1965).
49. D. J. Reish, and R. S. Carr, "The effect of heavy metals on the survival, reproduction, development, and life cycles for two species of polychaetous annelids," *Mar. Poll. Bull.* **9,** 24–27 (1978).
50. J. V. Gramlich, and D. E. Davis, "Effect of atrazine on nitrogen metabolism of resistant species," *Weeds* **15,** 157–160 (1967).
51. S. K. Ries, H. Chmiel, D. R. Dilley, and P. Filner, "The increase in nitrate reductase activity and protein content of plants treated with simazine," *PNAS* **58,** 526–532 (1967).
52. E. Ebert, and C. J. Van Assche, "Influence of atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) on auxin metabolism of plants," *Experientia* **25,** 758–759 (1969).
53. L. G. Copping, and D. E. Davis, "Effects of atrazine on chlorophyll retention in corn leaf discs," *Weed Sci.* **20,** 86–89 (1972).
54. L. G. Copping, D. E. Davis, and C. G. P. Pillai, "Growth regulator-like activity of atrazine and ametryne," *Weed Sci.* **20,** 274–277 (1972).
55. B. Singh, D. K. Salunkhe, and S. H. Lipton, "Growth and chemical responses of bean plants to sublethal concentrations of *s*-triazine compounds," *J. Horticult. Sci.* **47,** 441–448 (1972).
56. S. K. Ries, "Subtoxic effects on plants," in L. J. Audus, ed., *Herbicides: Physiology, Biochemistry, Ecology.* 2nd ed. (Academic Press, New York, 1976).
57. W. J. Allender, "The effect of sublethal concentrations of phytotoxins on plant growth," Doctoral dissertation, The University of Sydney, Australia (1995).
58. G. E. Walsh, and L. H. Bahner, "Toxicity of textile mill effluents to freshwater and estuarine algae, crustaceans and fishes," *Environ. Pollut. Ser. A. Ecol. Biol.* **21,** 169–179 (1980).
59. G. E. Walsh, K. M. Duke, and R. B. Foster, "Algae and crustaceans as indicators of bioactivity of industrial wastes," *Water Res.* **16,** 879–883 (1982).
60. E. J. Calabrese, and L. A. Baldwin, "Significant biological effects below the NOAEL in reproductive toxicology," *J. Appl. Toxicol.* (In press, 1999).
61. M. T. Elnabarawy, A. N. Welter, and R. R. Robideau, "Relative sensitivity of three daphnid species to selected organic and inorganic chemicals," *Environ. Toxicol. Chem.* **5,** 393–398 (1986).
62. C. W. M. Bodar, C. J. Van Leeuwen, P. A. Voogt, and D. I. Zandee, "Effect of cadmium on the reproduction strategy of *Daphnia magna,*" *Aquatic Toxicol.* **12,** 301–310 (1988).
63. Q. H. Pickering, and M. H. Gast, "Acute and chronic toxicity of cadmium to the fathead minnow (*Pimephales promelas*)," *J. Fish. Res. Bd. Can.* **29,** 1099–1106 (1972).
64. G. P. Daston, J. M. Rogers, D. J. Versteeg, T. D. Sabourin, D. Baines, and S. S. Marsh, "Interspecies comparisons of A/D ratios: A/D ratios are not constant across species," *Fund. Appl. Toxicol.* **17,** 696–722 (1991).
65. A. R. D. Stebbing, and A. J. Pomroy, "A sublethal technique for assessing the effects of contaminants using *Hydra littoralis,*" *Water Res.* **12,** 631–635 (1977).
66. C. L. Browne, and L. E. Davis, "Cellular mechanisms of stimulation of bud production in *Hydra* by low levels of inorganic lead compounds," *Cell. Tiss. Res.* **177,** 555–570 (1977).
67. P. L. Gordon, and F. L. McEwen, "Insecticide-stimulated reproduction of *myzus persicae,* the green peach aphid (Homoptera: Aphididae)," *Can. J. Entomol.* **116,** 783–784 (1984).
68. J. Founk, and R. J. McClanahan, "Foliar potato insect control," C.C.P.U.A. Res. Rep. pp. 122–123. (1970).
69. G. Ritcey, R. McGraw, and F. L. McEwen, "Insect control on potatoes in Ontario from 1973 to 1982," *Proc. Entomol. Soc. Ont.* **113,** 1–6 (1982).
70. D. T. Lowery, and M. K. Sears, "Stimulation of reproduction of the green peach aphid (Homoptera: Aphididae) by azinphosmethyl applied to potatoes," *J. Econ. Entomol.* **79,** 1530–1533 (1986).
71. D. T. Lowery, and M. K. Sears, "Effect of exposure to the insecticide azinphosmethyl on reproduction of green peach aphid (Homoptera: Aphididae)," *J. Econ. Entomol.* **79,** 1534–1538 (1986).
72. H. Hodjat, "Effects of sublethal doses of insecticides and of diet and crowding on *Dysdercus fasciatus* Sign. (Hem., Pyrrhocoridae)," *Bull. Entomol. Res.* **60,** 367–378 (1971).
73. D. J. Sutherland, F. D. Beam, and A. P. Gupta, "The effects on mosquitoes of sublethal exposure to insecticides. I. DDT, dieldrin, malathion and the basal follicles of *Aedes aegypti* (L)," *Mosquito News* **27,** 316–323 (1967).
74. H. J. Hueck, D. J. Kuenen, P. J. Den Boer, and E. Jaeger-Draafsel, "The increase of egg production of the fruit tree red spider mite (*Metatetranychus ulmi koch*) under influence of DDT," *Physiol. Comp.* **2,** 371–377 (1952).
75. D. J. Kuenen, "Influence of sublethal doses of DDT upon the multiplication rate of *Sitophilus granarius* (Coleopt. Curculionidae)," *Entomol. Exp. Appl.* **1,** 147–152. (1958).
76. J. G. Rodriguez, H. H. Chen, and W. T. Smith, Jr., "Effects of soil insecticides on beans, soybeans and cotton and resulting effect on mite nutrition," *J. Econ. Entomol.* **50,** 587–593 (1957).

77. E. G. Essac, S. El-Gogary, M. S. Abdel-Fatah, and A. M. Ali, "Effect of carbaryl, methyl parathion, and endrin on egg production and percent pupation of the egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.)," *Zeits. Angew. Entomol.* **71,** 203–270 (1972).
78. H. J. Ball, and P. P. Su, "Effect of sublethal dosages of carbofuran and carbaryl on fecundity and longevity of the female western corn rootworm," *J. Econ. Entomol.* **72,** 873–876 (1979).
79. P. Folker-Hansen, P. H. Krogh, and M. Holmstrup, "Effect of dimethoate on body growth of representatives of the soil living mesofauna," *Ecotox. Environ. Safety* **33,** 207–216 (1996).
80. T. S. K. Johansson, "Some physiological effects of sublethal doses of sodium fluoride on the confused flour beetle (*Tribolium confusum* Duval)," Thesis, University of Wisconsin (1947).
81. J. G. Morse, and N. Zareh, "Pesticide-induced hormoligosis of citrus thrips (Thysanoptera: Thripidae) fecundity," *J. Econ. Entomol.* **84,** 1169–1174 (1991).
82. J. G. Morse, "Agricultural implications of pesticide-induced hormesis of insects and mites," *Hum. Exp. Toxicol.* **17,** 266–269 (1998).
83. H. E. Kleczkowska, and F. R. Althaus, "Response of human keratinocytes to extremely low concentrations of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine," *Mut. Res.* **367,** 151–159 (1996).
84. H. C. Pitot, T. L. Goldsworthy, S. Moran, W. Kennan, H. P. Glauert, R. R. Maronpot, and H. A. Campbell, "A method to quantitate the relative initiating and promoting potencies of hepatocarcinogenic agents in their dose-response relationships to altered hepatic foci," *Carcinogenesis* **8,** 1491–1499 (1987).
85. U. Lutz, S. Lugli, A. Bitsch, J. Schlatter, and W. K. Lutz, "Dose response for the stimulation of cell division by caffeic acid in forestomach and kidney of the male F344 Rat," *Fund. Appl. Toxicol.* **39,** 131–137 (1997).
86. Office of Technology Assessment (OTA), *Cancer Testing Technology and Saccharin* (U.S. Government Printing Office, Washington, DC, 1977).
87. T. D. Downs, and R. F. Frankowski, "Influence of repair processes on dose-response models," *Drug Metab. Rev.* **13,** 839–852 (1982).
88. R. W. O'Gara, M. G. Kelly, J. Brown, and N. Mantel, "Induction of tumors in mice given a minute single dose of dibenz[*a,h*]anthracene or 3-methylcholanthrene as newborns. A dose-response study," *J. Natl. Cancer. Inst.* **35,** 1027–1042 (1965).
89. R. R. Cook, "Responses in humans to low level exposures," in E. J. Calabrese, ed, *Biological Effects of Low Level Exposures: Dose-Response Relationships* (Lewis Publishers, Boca Raton, FL, 1994) pp. 99–109.
90. M. P. Waalkes, S. Rham, C. W. Riggs, R. M. Bare, D. E. Devor, L. A. Poirier, M. L. Wenk, J. R. Henneman, and M. S. Balaschak, "Cadmium carcinogenesis in male wistar [Crl:(WI)BR] rats: Dose-response analysis of tumor induction in the prostate and testes and at the injection site," *Cancer Res.* **48,** 4656–4663 (1988).
91. A. F. Hanham, B. P. Dunn, and H. F. Stich, "Clastogenic activity of caffeic acid and its relationship to hydrogen peroxide generated during autooxidation," *Mut. Res.* **116,** 333–339 (1983).
92. IARC, "Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins," *IARC Monographs* **56,** 114–134 (1993).
93. S. Nesnow, J. Ross, G. Nelson, K. Wilson, B. Roop, A. Jeffers, A. Galati, G. Stoner, R. Sangaiah, and A. Gold, "Cyclopenta[*cd*]pyrene-induced tumorigenicity, Ki-*ras* codon 12 mutations and DNA adducts in strain A/J mouse lung," *Carcinogenesis* **15,** 601–606 (1994).
94. A. K. Prahalad, J. A. Ross, G. B. Nelson, B. C. Roop, L. C. King, S. Nesnow, and M. J. Mass, "Dibenzo[*a,l*]pyrene-induced DNA adduction, tumorigenicity, and Ki-*ras* oncogene mutations in strain A/J mouse lung," *Carcinogenesis* **18,** 1955–1963 (1997).
95. R. J. Kociba, D. G. Keyes, J. E. Beyer, R. M. Carreon, C. E. Wade, D. A. Ditenber, R. P. Kalnins, L. E. Frauson, C. N. Park, S. D. Barnard, R. A. Hummel, and C. G. Humiston, "Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats," *Toxicol. Appl. Pharmacol.* **46,** 279–303 (1978).
96. R. B. Conolly, and M. E. Andersen, "Hepatic foci in rats after diethylnitrosamine initiation and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin promotion: evaluation of a quantitative two-cell model and of CYP 1A1/1A2 as a dosimeter," *Toxicol. Appl. Pharmacol.* **146,** 281–293 (1997).
97. S. C. Tripathi, K. A. Vosseller, and D. L. McCormick, "Arachidonic acid metabolism and cell proliferation in rat mammary carcinoma cells treated with indomethacin," *Biochim. Biophys. Acta* **1316,** 5–7 (1996).
98. R. D. Moore, and T. A. Pearson, "Moderate alcohol consumption and coronary artery Disease: a review," *Medicine* **65,** 242–267 (1986).
99. A. G. Shaper, G. Wannamethee, and M. Walder, "Alcohol and mortality in British men: Explaining the U-shaped curve," *Lancet* **283,** 179–186 (1988).
100. M. Maclure, "Demonstration of deductive meta-analysis: Ethanol intake and risk of myocardial infarction," *Epidemiol. Rev.* **15,** 1–24 (1993).
101. J. M. Gaziano, "Alcohol and coronary heart disease," *BELLE Newslett.* **4,** 1–5 (1995).
102. F. C. Ballantyne, R. S. Clarck, H. S. Simpson, and D. Ballantyne, "High density and low density lipoprotein subfractions in survivors of myocardial infarction and in control subjects," *Metabolism* **31,** 433–437 (1982).
103. G. J. Miller, F. Hammet, S. Saltissi, S. Rao, H. VanZeller, J. Coltart, and B. Lewis, "Relation of angiographically defined coronary artery disease to plasma lipoprotein subfractions and apolipoproteins," *Brit. Med. J.* **282,** 1741–1744 (1981).
104. W. L. Haskell, C. Camargo, P. T. Williams, K. M. Vranizan, R. M. Kraus, F. T. Lindgren, and P. D. Wood, "The effect of cessation and resumption of moderate alcohol intake high-density-lipoprotein subfractions," *New Engl. J. Med.* **310,** 805–810 (1984).
105. J. M. Gaziano, J. E. Buring, J. L. Breslow, S. Z. Goldhaber, B. Rosner, M. VanDenburgh, W. Willett, and C. H. Hennekens, "Moderate alcohol intake, increased levels of high density liporportein and its subfractions, and decreased risk of myocardial infarction," *New Engl. J. Med.* **329,** 1829–1834 (1993).
106. E. J. Calabrese, "Hormesis revisited: New insights concerning the biological effects of low-dose exposures to toxins," *Environ. Law Report.* **27,** 10526–10532 (1997).
107. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), *Sources and Effects of Ionizing Radiation: Report to the General Assembly, with Scientific Annexes. Annex B* (1994) pp. 185–272.
108. S. Wolf, and G. Olivieri, eds, "Symposium—Adaptive response and radiation," *Mutat. Res.* **358,** 125–243 (1996).
109. C. O. Townsend, "The correlation of growth under the influence of injuries," *Ann. Bot.* **11,** 5-9-532 (1897).
110. P. Hofmann, "Ueber die Gultigkeit des Arndt-Schulzschen biologischen Grundgesetzes bei der Wirkung von Bakteriengiften," Inaugural Dissertation, tierarztl. Fakultat. Munchen (Translated from the German by University of Massachusetts translation services, 1922).
111. S. E. Branham, "The effects of certain chemical compounds upon the course of gas production by baker's yeast," *J. Bacteriol.* **18,** 247–264 (1929).
112. E. C. Smith, "Effects of ultra-violet radiation and tempera-

ture on fusarium. II. Stimulation," *Bull. Torrey Bot. Club* **62,** 151–164 (1935).
113. E. J. Calabrese, "Evidence that hormesis represents an 'overcompensation' response to a disruption in homeostasis," *Ecotoxicol. Environ. Safety* **42,** 135–137 (1999).
114. E. J. Calabrese, and R. Blaine, "A single exposure to many carcinogens can cause cancer," *Environ. Law Rep.* **28,** 10254–10262.
115. E J. Calabrese, and L. A. Baldwin, "A general classification of U-shaped dose-response relationships in toxicology and their mechanistic foundations," *Hum. Exp. Toxicol.* **17,** 353–364 (1998).
116. G. Fiskesjo, "The allium test—An alternative in environmental studies: The relative toxicity of metal ions," *Mutat. Res.* **197,** 243–260 (1988).
117. A. V. Subhadra, A. K. Nanda, P. K. Behera, and B. B. Panda, "Acceleration of catalase and peroxidase activities in *Lemna minor* L. and *Allium cepa* L. in response to low levels of aquatic mercury," *Environ. Pollut.* **69,** 169–179 (1991).
118. P. Vichi, and T. R. Tritton, "Stimulation of growth in human and marine cells by adriamycin," *Cancer Res.* **49,** 2679–2682 (1989).
119. S. K. Ries, and A. Gast, "The effect of simazine on nitrogenous components of corn," *Weeds* **13,** 272–274 (1965).
120. S. K. Ries, R. P. Larsen, and A. L. Kenworthy, "The apparent influence of simazine on nitrogen nutrition of peach and apple trees," *Weeds* **11,** 270–273 (1963).
121. C. R. Thompson, and G. Kats, "Effects of continuous H_2S fumigation on crop and forest plants," *Environ. Sci. Technol.* **12,** 550–553 (1978).
122. R. B. Laughlin Jr, J. Ng, and H. E. Guard, "Hormesis: A response to low environmental concentrations of petroleum hydrocarbons," *Science* **211,** 705–707 (1981).
123. S. Koivisto, M. Ketola, and M. Walls, "Comparison of five cladoceran species in short- and long-term copper exposure," *Hydrobiologia* **248,** 125–136 (1992).
124. A. R. D. Stebbing, and G. W. Heath, "Is growth controlled by a hierarchical system?" *Zool. J. Linn. Soc.* **80,** 345–367 (1984).
125. J. E. Cassida, and T. C. Allen, "A laboratory method for evaluating the phytotoxicity or phytostimulation of insecticides," *Science* **13,** 553–555 (1951).
126. C. T. DeRosa, H. R. Pohl, M. Williams, A. A. Ademoyero, C-H. S. J. Chou, and D. E. Jones, "Public health implications of environmental exposure," *Environ. Health Perspect.* **106** (suppl. 1), 369–378 (1998).
127. E. J. Calabrese, "Expanding the RfD concept to incorporate and optimize beneficial effects while preventing toxic responses from non-essential toxicants," *BELLE Newslett.* **4,** 1–10 (1996).
128. D. Gaylor, "Safety assessment with hormetic effects," *BELLE Newslett.* **6,** 6–8 (1998).
129. E. J. Calabrese, and L. A. Baldwin, "Hormesis as a default parameter in RfD derivation," *Hum. Exp. Toxicol.* **17,** 444–447 (1998).
130. J. Cotruro, and M. Regelski, "National primary drinking water regulations for volatile organic chemicals," in E. J. Calabrese, C. E. Gilbert, and H. Pastides, eds, *Safe Drinking Water Act: Amendments, Regulations and Standards,* (Lewis Publishers, Chelsea, MA, 1989) pp. 29–34.
131. B. L. Cohen, "Test of the linear-no threshold theory of radiation carcinogenesis for inhaled radon decay products," *Health Physics* **68,** 157–174 (1995).
132. K. T. Bogen, "Do U.S. county data disprove linear no-threshold predictions of lung cancer risk for residential radon?—A preliminary assessment of biological plausibility," *Hum. Ecol. Risk Assess.* **3,** 157–186 (1997).