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Thresholds for carcinogens

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ABSTRACT

Current regulatory cancer risk assessment principles and practices assume a linear dose-response relationship—the linear no-threshold (LNT) model—that theoretically estimates cancer risks occurring following low doses of carcinogens by linearly extrapolating downward from experimentally determined risks at high doses. The two-year rodent bioassays serve as experimental vehicles to determine the high-dose cancer risks in animals and then to predict, by extrapolation, the number of carcinogen-induced tumors (tumor incidence) that will arise during the lifespans of humans who are exposed to environmental carcinogens at doses typically orders of magnitude below those applied in the rodent assays. An integrated toxicological analysis is conducted herein to reconsider an alternative and once-promising approach, tumor latency, for estimating carcinogen-induced cancer risks at low doses. Tumor latency measures time-to-tumor following exposure to a carcinogen, instead of tumor incidence. Evidence for and against the concept of carcinogen-induced tumor latency is presented, discussed, and then examined with respect to its relationship to dose, dose rates, and the dose-related concepts of initiation, tumor promotion, tumor regression, tumor incidence, and hormesis. Considerable experimental evidence indicates: (1) tumor latency (time-to-tumor) is inversely related to the dose of carcinogens and (2) lower doses of carcinogens display quantifiably discrete latency thresholds below which the promotion and, consequently, the progression and growth of tumors are delayed or prevented during a normal lifespan. Besides reconciling well with the concept of tumor promotion, such latency thresholds also reconcile favorably with the existence of thresholds for tumor incidence, the stochastic processes of tumor initiation, and the compensatory repair mechanisms of hormesis. Most importantly, this analysis and the arguments presented herein provide sound theoretical, experimental, and mechanistic rationales for rethinking the foundational premises of low-dose linearity and updating the current practices of cancer risk assessment to include the concept of carcinogen thresholds.

1. Introduction

The risk assessment process for carcinogens is currently based on the induction of tumors in animals at high doses coupled with a downward linear extrapolation across doses—often over three to seven orders of magnitude—in order to approximate safe exposures at environmentally low doses [17,18,25,32,36]. The goal of this high-dose testing process is to provide regulatory agencies with estimates of cancer risks that can then be fine-tuned by policy makers after considering the possible effects of proposed carcinogen standards on the economy, public health, technology, and values of society. Such assessments usually result in acceptable risks of 10^{-5} (i.e., one in one-hundred thousand) or lower. Currently, cancer risk assessments of low-level carcinogen exposures are predicated on uncertain extrapolations of bioassay data, require unverifiable and often error-prone human decision-making, and depend on a flawed linear no-threshold (LNT) dose-response model for accurate estimates of low dose cancer risks [1,14,26,33,72,82,88,95,100]. Given the uncertainties inherent in such a process, alternative cancer risk-assessment strategies would seem to be most warranted. Important results from archived toxicology studies as well as from more contemporaneous findings indicate that the magnitude of the carcinogen dose is inversely related to tumor latency or time-to-tumor (i.e., low doses yield long latencies and vice versa) and that such a relationship may provide the foundations for an alternative and improved approach to cancer risk assessment. This paper revisits, reconsiders, and integrates the available theoretical, experimental, and mechanistic evidence for adopting a

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cancer risk assessment strategy based on the concept of tumor latency and the existence of an apparent carcinogen threshold.

Nearly 80 years ago, two researchers independently studied and published the effects of dose on tumor latency. One was Harold F. Blum from the USA, who studied the effects of ultraviolet (UV) radiation on skin cancer in mice at the National Cancer Institute (NCI), and the other was Hermann Druckrey from Germany, who studied the effects of genotoxic chemical carcinogens on rats. Both Blum and Druckrey independently observed that tumor latency increases as the dose decreases. Based on their research, both scientists suggested that it was possible to decrease the dose of a carcinogen to a point below which individuals would die of other ailments long before succumbing to cancer. Furthermore, both hypothesized that the extension of tumor latency to beyond a normal lifespan could define a low "safe" dose, and that such low "safe" doses resulted from their diminishing effects on "rates" of tumor promotion.

As described subsequently, these striking early observations, and the further promise from important follow up research, became victims of World War II as Blum, then at the US National Cancer Institute (NCI), was redirected to another health-related research area, and Druckrey was arrested in Germany and placed in Allied custody for a period before he was eventually permitted to resume his professional career. The present analysis explores their independent but related contemporary research on the dose-tumor latency relationship, as well as its potential for use in updating and improving current cancer risk-assessment practices. Since the time of Blum and Druckrey, however, other relevant biomedical and toxicological advancements have occurred that provide greater depth, understanding, and perspective of the dose-tumor latency relationship and those issues have been integrated into the present analysis.

2. Initial studies on tumor latency: dose, dose rate, and dose response

That UV radiation could induce skin cancer was first shown by Findlay [44] who reported the occurrence of papillomata and malignant epitheliomas in albino mice, then subsequently in rats [45]. During the next five years, other research groups who were unaware of the Findlay findings conducted experiments on UV-induced skin cancer in mice that confirmed all of Findlay's cancer observations for wavelengths shorter than 320 nm (0.32 μm) [8,9,54,74,84].

Following the creation of the NCI in 1938, Blum and several colleagues set out to assess the occurrence and reproducibility of several key quantitative features of UV-induced skin tumors on the ears of Strain A male mice. Besides designing an experimental animal protocol offering reduced variability, the research benefited from newly refined dose-delivery methods and a very low rate of mouse mortality that enabled tumors to develop fully. Nevertheless, despite these efforts of variability reduction, the inter-individual variability for the occurrence of skin cancer in this study was considerable. For example, daily treatment with high doses led to the occurrence of first tumors at about 100 days, with 50% of the test mice showing tumors by five months (~150 days) and all mice showing tumors by eight months (~240 days). Blum [7] reported that differences in dose (i.e., ergs/cm^2), intensity (i.e., dose rate in ergs/cm^2/sec), or intervals between treatments, did not affect the slope of the dose response, but only the duration of the latency period. Those animals receiving a lower dose, less intensity, longer interval, or various combinations of those parameters, displayed longer latency periods that were quantifiable within an integrated biomathematical framework. Of further interest is that the various dose-intensity linked responses could be superimposed on one another by their integration along the dose and time axes [7]. The occurrence of skin cancer on the ears of Strain A male mice used in the Blum studies was very low. Historical control data indicated only five tumors in 8000 mice [12].

Of theoretical and practical importance is that the mortality of the Strain A mice was quite low, permitting tumors to develop over the lifespan. In general, all mice tended to develop tumors before death in the high-dosage groups.[1] However, for experiments in the low-dose range, significantly fewer mice developed tumors prior to their deaths from natural causes. This dose-dependent progression of onset for UV-induced cancers led to the conclusion that "all the mice would develop tumors, given a long enough life span since there is no determinable threshold for the induction of these tumors. That is, we had to think that cancers develop in all the treated mice, but that in some, development of the tumor is so slow that it does not appear within the lifetime of the animal." (page 194 [8]). While this argument supports a linear dose-response model, it also supports the concept of a practical or effective threshold due to the relationship between dose and tumor latency.

Despite the reasonable articulation of Blum above, he also directly tested [9–11] the concept of LNT via a dose-rate methodology, which he referred to as a type of "reciprocating" experiment (i.e., studies that give the same total dose but at different dose rates). In this case, different groups received the same total dose of UV radiation, but it was given over widely differing durations that ranged from relatively short to very long. If the LNT were to be supported, the tumor incidence should be the same, with the genetic damage being cumulative and irreversible. At dose rates above 0.35×10^4 erg/cm^3/sec, the reciprocal tended to hold within the statistical constraints of the experiments. However, the tumor response fell markedly below the linearly predicted decline in response as the UV doses decreased. These experiments, therefore, did not support the LNT dose-response model for UV radiation within the low-dose range. Although Blum would conclude that the LNT model and the dose-latency relationship were both valid, he would also conclude that in the case of LNT such validity was restricted to a definable dose-rate range [7,12].

The Blum findings were important because they demonstrated an experimental animal system that was reproducible and could be used to establish quantitative relationships amongst doses, intensities, intervals, and durations of exposures for both tumor incidence and time-to-tumor outcomes. Furthermore, experimental parameters were varied to test specific hypotheses probing the effects of tumor latencies on tumor incidences. The experiments revealed that linearity occurred at the higher but not the lower doses and dose rates, and that tumor latency was a mathematical inverse function of dose, intensity, and interval. In general, the tumor-latency interval increased as exposure decreased (Fig. 1). Although this relationship could become complex when integrating multiple parameters of dose, intensity, and interval, Blum indicated that such a relationship was inversely proportional to the square root of the dose rate. However, he also observed that this inverse relationship disappears at high doses and dose rates [8–11] but becomes ever more apparent at lower doses and dose rates.

The "reciprocity" experiments (i.e., studies that give the same total dose but at different dose rates) by Blum were critically important because they enabled testing of the LNT assumption across a broad range of doses. Blum's studies were published in 1943, just prior to the publication of a dose-rate study on X-ray-induced mutations in fruit flies [80], of which Blum was unaware. Publication of the Ray-Chaudhuri

[1] The most complete data sets from the Blum studies employed five exposures per week that were often carried out until treatment groups displayed at least 50% tumors. The treatment groups usually had between 50 and 100 animals per data point. Blum would also try to push the experimental limits of dosing at the low and high ends of exposure. At the high end this would be compromised by toxicity and at the low exposure end, the tumors would occur so late in appearance that the average life span of the mice would be reached prior to a significant number of tumors developing [13].

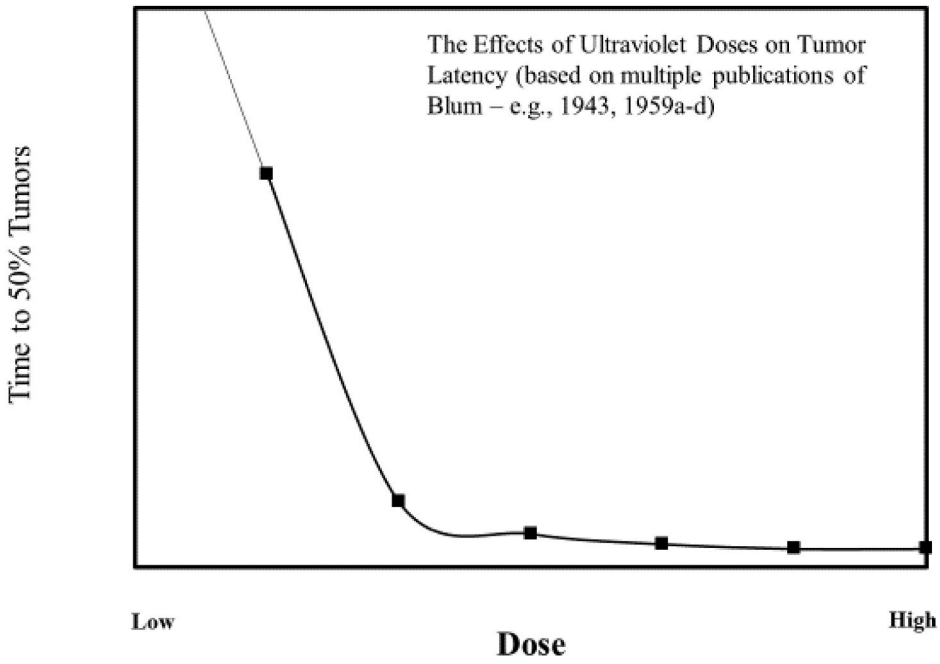

Fig. 1. The effects of ultraviolet doses on tumor latency.

study had been delayed several years and was conducted with the guidance of Hermann Muller, a Nobel Laureate and ardent advocate of LNT (See Ref. [30] for critique of [80]). Blum and the team of Muller and Ray-Chaudhuri had independently realized the practicality of using the dose-rate "reciprocity" model for testing the LNT hypothesis. With his findings, Blum [7,8], was able to establish the following major concepts:

(1) The linear dose response appeared valid at high doses.
(2) The latency period is inversely related to dose, intensity, and interval, and it can be quantitatively estimated.
(3) Tumor incidence is dependent on dose rate, with the incidence dropping off markedly at lower dose rates. This observation reveals that at lower dose rates the LNT concept becomes progressively less valid while, at the same time, the threshold model becomes more valid.
(4) These findings led Blum to suggest the possibility of a cellular repair process at low doses/dose rates.

Interestingly, these tumor findings and observations by Blum would precede by 14 years the seminal study of Russell et al. [85] on dose-rate effects of X-rays on mutations in mouse spermatogonia. Even though the Russell mutation findings on dose rates would essentially support Blum's tumor conclusions on dose rates, Russell never cited Blum in the 1958 publication or in any other of his subsequent publications on dose rates.

On December 4, 1959, one year after the publication by Russell et al. [85] in *Science* on dose rate effects of X-rays on mutations, Blum published a summary article in *Science* on dose-rate effects of UV radiation on cancer [10]. In this publication, Blum stated that "to the best of my knowledge there are no data on experimental carcinogenesis by ionizing radiation that have been obtained in a manner comparable to those with ultraviolet light, nor that will permit a comparable analysis and until such data have been obtained it seems wisest to accept the parallel", i.e., assumed similar responses for the two types of radiation. (Page 1547, column 1- [10]). Although Blum's statement is historically and technically accurate when implying the importance of his dose-rate experiments to carcinogenesis, it could be argued that the earlier findings of Russell et al. [85] were equally important to the field of mutagenicity. Specifically, Russell and co-workers rigorously established dose rate as a valid dose-response concept that affects mutational outcomes and, as such, it should have been duly acknowledged by the larger regulatory community as a paradigm-altering concept that not only challenges the LNT model but also significantly affects the theoretical and practical aspects of both genetic and cancer risk assessments [22,23]. In fact, it was late December of 1958, and only about 10 days after Russell's *Science* publication, that the National Committee for Radiation Protection and Measurement (NCRPM) (1960) recommended, for the first time, adopting the LNT model for cancer risk assessment [66].

In his 1959c *Science* publication [10], Blum stated that his dose-response relationship (Fig. 1, *Science*, 1959c) provided no evidence of a "threshold" below which cancer is not induced. "On the contrary, they concluded that any dosage may induce cancer in some fraction, however small, of the population." However, Blum would seemingly contradict this statement in the same paper, noting that there was evidence of recovery/repair from a carcinogenesis process induced by UV. Such repair suggested the possibility that "a threshold exists at some very low level." (page 1546).

In his *Science* publication, Blum neglected to cite his previous tumor studies (described above) that provided evidence for "reciprocity" at high doses in mice (thus supporting LNT at high doses) but provided no evidence for "reciprocity" at critical lower doses (thus not supporting LNT at low doses). Two other "reciprocity" studies were conducted on carcinogens that used mutations instead of tumors as their experimental endpoints. In these two studies, low-dose "reciprocity" could be demonstrated neither in mice, as shown by Russell et al. [85], nor in fruit flies, as reported by Caspari and Stern [31] and as detailed later by Calabrese [19]. Like Blum's [7] study, these two "reciprocity" studies

had also failed to support the use of LNT for carcinogens in the low-dose zone.

The findings of Blum and his colleagues are historically important since they address key methodological issues involved in assessing cancer risks from UV exposures and they also examine the relationships of dose to latency and dose rate to total dose. The accuracy and credibility of the Blum findings are a direct result of the substantial statistical power embedded into the analytical framework of his studies, the robust study designs, the powerful dosing regimens, and the extensive effort to minimize response variability.

Although the findings of Blum are viewed as conceptually highly significant, they appeared to have limited impact on his research field at the time, as judged by his citations and applications. Such apparent "under" recognition of the Blum findings may have been at least partly due to the redirection of his research into other areas deemed more urgent to the prosecution of World War II. Blum would return to the dose-tumor latency issue nearly two decades later with a summary publication of his earlier research in *Science* [8] and then in a monograph [9].

Taken together, these archived "reciprocity" studies indicate that the biological response is different when the same total radiation dose is given at different rates, i.e., threshold responses were observed at lower dose rates and linear responses at the higher rates. After more than six decades of progress in radiobiological research, it is now commonly recognized that innate protective systems exist in the body and its cells to prevent, repair, and remove radiation damage efficiently, assuming of course the protective/repair capacities of these systems are not overtaxed by radiation at a dose or a dose rate that is too high and basically incompatible with life [43]. It would, therefore, seem reasonable to expect different biological outcomes (threshold versus linearity) depending on whether the same total dose would be delivered as one short, single acute exposure (within seconds to minutes), as a series of fractionated doses (smaller acute doses repeated at intervals), or as a continuous long exposure at a low, fixed dose rate. Recently, it has been shown that these innate protective/repair systems not only exist but are adaptive and can be induced to function at higher-than-normal efficiencies if they are first preconditioned with a low, subtoxic dose of radiation [68,43]. Even more recently, however, these adaptive/protective responses have, in fact, been shown to be hormetic responses [20,21] and, as such, are mediated (at least in part) by redox-activated Nrf2 pathways that actually enhance the radio-resistance of cells [29]. (A fuller discussion of Nrf2-mediated hormesis and its relationship to carcinogen thresholds and tumor latencies is found in section 5.4.)

Given the historical progression of experimental findings above, it may be helpful—as a theoretical exercise—to classify cells into one of three categories based on the type of protective/repair systems that scientists have assumed cells possessed when arguing for their preferred dose-response model. Historically, the protective/repair systems of cells were assumed to be either (1) "nonexistent", (2) "constitutive", or (3) "adaptive". Cells with "nonexistent" or no protective/repair systems would obviously be most vulnerable and would likely adhere to a linear dose-response model, whereby any amount of radiation damage, no matter how small, was assumed to be irreparable, cumulative, and thus potentially deadly. On the other hand, cells with "constitutive" protective/repair systems would have a fixed capacity to respond to radiation damage and thus afford limited protection, reconciling favorably with the threshold dose-response model. Finally, cells having "adaptive" systems of protection and repair would be able to respond to low doses of radiation by actually enhancing their pre-adaptive capacities to better protect, repair, and survive, thereby conforming to the biphasic characteristics of the hormetic dose-response model. The questions to ask now are twofold. First, which of these dose-response models is and will be best supported by experimental, theoretical, and mechanistic research findings at the cellular and subcellular levels? Second, which model best fits into an evolutionary perspective and can explain how life was, is, and will be able to evolve and therefore adapt to perpetual environmental stressors on this planet?

3. Expanding the concept of latency: incidence vs latency, threshold vs LNT, and the quantification of latency

The principal way in which dose is employed to assess carcinogenic risk is to determine the incidence of tumors (i.e., the number) induced in rodents following a two-year experimental treatment protocol (i.e., a bioassay) with several relatively high doses of a selected carcinogen, thereby determining the dose-response relationship between dose and tumor incidence. In 1943, however, Hermann Druckrey conducted the first in a long series of tumor studies [36] to determine the dose-response relationship between dose and tumor latency (i.e., the time required for tumors to appear after treatment with a single dose). Essentially, Druckrey introduced a new methodology to estimate carcinogenic risk that was complementary to the standard methodology for determining the relationship between dose and tumor incidence. In addition, Druckrey judiciously selected experimental test agents that had already demonstrated high carcinogenic potency and low acute toxicity in the two-year rodent bioassay. These agents included nitrosamines and the related dialkylaminobenzenes and dialkylaminostilbenes. The incidence of tumors induced by these agents at high doses was usually so high that the delay or lag in time-to-tumor appearance (i.e., the latency) provided a good quantitative index of the relative carcinogenic potential of each test agent. Thus, both a novel study design and a wise choice in test agents enabled Druckrey [37,38] to demonstrate that tumor latency varies inversely as a fractional power of the carcinogen dose. Before Druckrey's innovative testing protocol had been established, however, it was not practically possible to obtain adequate quantitative data on tumor incidence across a broad range of low doses with any reliable precision. As a result, the testing of low doses was generally limited to a very narrow range of low doses (in an attempt to reduce variability) and focused on the appearance of only a few tumors, while completely ignoring tumor latency.

At the time, Druckrey strongly adhered to the idea that the dose response for chemical carcinogens, such as the nitrosamines, was linear with respect to tumor incidence. At the very foundation of linearity was the notion that a single unit of dose of a specific carcinogen would produce some constant amount of irreversible (irreparable) cellular damage, which, in turn, would yield a constant and irreversible tumorigenic response. Thus, given the irreversibility and constancy of the tumor response on a per-unit-of-dose basis, the total tumor response should logically reflect the summation of all responses on a per-unit-of-dose basis over time. Graphically, such linearity implies that the tumor response (on the y axis) occurs at a "constant" probability (constant slope) per unit of delivered dose, no matter the size of the total delivered dose (on the x axis), large or small. In other words, linearity means that one unit of either a high or a low dose (i.e., on a per-unit-of-dose basis) will elicit quantitatively and qualitatively equivalent (the same) tumor responses, even though the aggregate responses at higher total doses obviously will be proportionately greater than the aggregate responses at lower total doses. For linearity then, tumor responses are the same on a per-unit-of-dose basis, are irreversible, and accumulate over time such that the aggregate tumor response is dependent on and proportional to the total delivered dose. It is important to note, however, that this linear theory, as embodied in the LNT model, remains highly contested today. In fact, many studies involving the endpoint of tumor incidence (e.g. [27,47,58,60,97]) are strikingly inconsistent with the LNT, supporting

an agent-specific threshold value in the low-dose range whereby cells can detoxify carcinogens and/or repair carcinogen-induced damage to prevent and/or delay the tumorigenic process.

What about the relationship between dose and time-to-tumor (tumor latency), and what can the studies of Blum and Druckrey reveal about this relationship? Essentially, Blum and Druckrey, as discussed earlier, independently showed that tumor latencies for numerous carcinogens are inversely dependent on dose, with lower doses generating longer latencies and higher doses generating shorter latencies. However, at some sufficiently low threshold dose—and for all doses below this threshold dose—tumors fail to materialize during the median normal lifespan of the animals, indicating that an "effective" threshold dose exists for tumor latency whereby all subthreshold doses would yield "virtual" tumors that arise only if the animals could live long enough. Thus, the failure of the subthreshold doses to generate palpable tumors during a normal lifespan implies one of two hypothetical alternatives: either (#1) tumorigenesis is delayed to the extent that tumors would materialize only after a latency that extends beyond the lifespan of the animal or (#2) tumors would never materialize, either as real tumors during or as "virtual" tumors beyond one's lifespan. The first hypothetical alternative (#1) affirms the observations of Blum and Druckrey, indicating that the time-to-tumor is dependent on and inversely related to dose, except for subthreshold doses whereby tumor latencies extend beyond lifespan to yield only "virtual" tumors. (Note: Whether and to what extent the relationship between supra-threshold doses that yield real tumors and tumor latency is linear or nonlinear is a critical question that will be addressed later in this paper.) The second hypothetical alternative (#2) implies that the subthreshold doses do not extend tumor latency beyond normal lifespan, do not delay the tumorigenic process, and yield neither "real" nor "virtual" tumors, even if tumor latency could extend to infinity. If this were true, then the threshold dose for tumor latency would be a "real" instead of "effective" threshold, whereby no "real" or "virtual" tumors would ever be produced unless the "real" threshold is exceeded.

The fact that tumorigenesis is a very complex series of multiple cellular and molecular events that are integrated and coordinated over a relatively long duration implies that any number of disruptions (e.g., repair processes or activated immune systems) could delay tumorigenesis beyond lifespan, as per hypothetical alternative #1, or even prevent tumor expression indefinitely, as per hypothetical alternative #2. With respect to the process of cancer risk assessment, as presently conducted, however, knowing whether the threshold for tumor latency is "real" or "effective" is immaterial, academic, and of no practical importance.

The theoretical extrapolation of tumor latency to beyond lifespan may be used not only to define discrete threshold values (doses) relative to controls but also to quantify and estimate specific cancer risks of subjects exposed to specific levels of environmental carcinogens. A key step toward in this quantification of risk involved Druckrey, who reported that dose times (X) latency raised to the power "n" (dose x latency)^n proved to be a constant when a characteristic value of n is determined. Druckrey referred to this "constant" as the "index of carcinogenicity", using this value to compare the relative carcinogenic potency of chemically related carcinogens. The lower the number of the constant (i.e., the lower the effective dose and/or the shorter time to detectable tumor) the more potent the carcinogen. The chemical agents that he studied displayed different constants but generally similar values for the exponent. Based on his research with nitrosamines, Druckrey [38] derived the following mathematical relationship:

$$d \times T_{50}^{n} = C$$

where d is the daily dose rate, T_{50} is the time from the first exposure to the occurrence of tumors in 50% of the animals, n is a positive exponent (usually n approximates 3.0) and C is a constant. In his extensive experimentation, Druckrey demonstrated that a lognormal time-to-tumor distribution tended to fit the experimental data well.

Jones and Grendon [55] transformed Druckrey's concept to explain latency rather than relative carcinogenicity. This was accomplished by expressing Druckrey's relationship but equating a constant over the nth root of d. Log + then is a constant −1/n log d. This relationship is a straight line with a slope equal to −1/n. This enabled Grendon and Jones to compare latency periods for different doses (D) of any carcinogen. The relationship was expressed as:

$$t = t_0 \, (D_0/D)^{1/n}$$

D_0 is the dose producing a tumor with a latency period (t_0) and the usual value of n is 3.

Jones and Grendon [55] seized upon this concept and reanalyzed the findings of Druckrey and other research groups as well. They came to the conclusion that the mean time t from the first exposure until the occurrence of tumors was inversely related to about the 1/3rd power of the dose as shown in the equation above. In order to move this mathematical relationship to the biological domain, they proposed the following explanation: the carcinogen exposure would create a uniform density of initiated cells in the affected organ and the transformed cells would lead to tumor formation only when two or more clones derived from similarly affected cells grow enough such that they come in contact with each other.[2] They then postulated that if the density of the original transformed cells is proportional to dose, then the mean distance between any two adjacent transformed cells would also be inversely proportional to dose. If this is the case, then the average distance between any two adjacent latency cells would be proportional to the cube root of the dose. Going further, they stated that if the rate of growth of the individual precancerous clones is uniform, then the mean value of the time required for the adjacent clones to reach and join each other would be inversely related to the cube root of the dose of the carcinogen.

As noted above, the key feature of this proposal was that at sufficiently low doses of carcinogen (i.e., subthreshold for tumor latency), tumor formation was predicted to occur long after the individual had died of natural causes. Thus, in the reanalysis of the studies from Druckrey and others, Jones and Grendon mathematically confirmed the experimental observations of Druckrey and Blum and identified threshold doses with tumor latencies beyond the normal lifespan of the controls. The calculations used to identify threshold doses and predict time-to-tumor beyond lifespan, as mentioned above, provide potential mathematical approaches for developing novel cancer risk assessment strategies.

The key result was the demonstration of a consistent inverse relationship between dose and tumor latency, whereby a low, subthreshold dose yields a longer than lifespan time-to-tumor. In an analytical sense, the groups treated with low, subthreshold doses would have tumor incidences indistinguishable (i.e., not significantly different) from controls. An attractive aspect of this new framework was that one could accept, in theory, the linear dose-response model and yet still derive a "practical threshold" for carcinogens. The proposal of Druckrey represented a toxicologically based integration of the LNT and threshold dose response models. In practical terms, Jones and Grendon [55] found that

[2] The tumor development mechanism proposed by Jones and Grendon [55] is a direct off-shoot of the proposal of Fisher and Halloman [46] which used observations of age-dependent stomach cancer death incidence to develop a novel biostatistical model for cancer risk assessment. The cancer incidence was estimated to occur with the 5th or 6th power of age. The power law relationship could be explained assuming there were 6 or 7 different cells that were mutated/transformed within a single tissue, thereby initiating the tumor response. These transformed cells were then assumed to become integrated, developing into a tumor mass. This tumor model concept was subsequently transformed into the polyclonal model or multi-cell theory of carcinogenesis. This hypothesis developed support within the cancer biology community as seen in the writings of Wright and Peto [99], Jones and Grendon [55] and recent expansion of data supporting this model by Parsons [70,71].

if the dose were reduced by a factor of 1000 in the linear zone, the latency period would increase by about a factor of 10. For tumors with prolonged latencies the estimated duration would far exceed the human lifespan.

The Druckrey "dose-latency-and-tumor-incidence" concept generated substantial interest worldwide. Several researchers have validated the findings of Druckrey (e.g., Ref. [94]). In the former Soviet Union, the concept of "dose latency" was adopted in risk assessment practices by the late 1970s using experimental studies by Yanysheva and Antomonov [101] for the potential carcinogen benzo(a)pyrene. These authors indicated that any carcinogenic effect of the benzo(a)pyrene would be "seen" on a theoretical basis only long after the normal lifespan of the test animal had been exceeded. The Druckrey concept was also applied to areas of occupational cancers by Enterline [39], as seen in a meta-analysis of 11 major epidemiological studies on asbestos exposure, for radium exposure by Evans [42], and for various radiation studies by Raabe [76,77,79].

Additional epidemiologic support for the dose-latency concept was reported by researchers of the Atomic Bomb Casualty Commission (ABCC) [6]. Five to eight years after the 1945 atomic bomb explosions in Nagasaki and Hiroshima (1950–1953), a peak increase in the incidence of leukemia was hypothesized to have occurred that reflected exposure to a mean estimated dose of 230 rem (2.3 Sv) of radiation. However, thirteen to nineteen years after the explosions (1958–1964), a second wave of leukemia incidence occurred that was associated with exposure to an estimated dose of only 30 rem (300 mSv). According to Jones and Grendon [55], the slope of the dose-time response closely fit the inverse cube root of the dose response relationship, further supporting the Druckrey hypothesis.

The dose-latency hypothesis of Jones and Grendon [55] was based upon a series of cancer bioassays involving carcinogenic polynuclear aromatic hydrocarbons (PAHs), nitrosamines, and ionizing radiation, including radionuclides and X-rays. The experiments used protocols involving either single exposures or daily exposures to a carcinogen. Of the nine (9) carcinogens presented in Figure 2a of Jones and Grendon [55], five (5) were evaluated according to single exposure protocols. All doses were tumorigenic, and all dose-response relationships were linear. To induce tumor growth with a single dose and to compensate for the absence of massive endogenous or exogenous tumor promoters, it was necessary to apply quite high doses in all of these experiments to induce both initiation (i.e., mutational effects) and tumor-promoting activities.

4. Public debate on latency: regulatory agencies and critics

The Jones and Grendon [55] study was published during a time of considerable environmental, political, and regulatory uncertainty in the USA over the development of a national risk assessment policy on carcinogens [83]. The Environmental Protection Agency (EPA) and the Occupational Safety and Health Administration (OSHA) were expected to develop this policy. OSHA took the lead and conducted multiple national policy hearings in 1978 to examine the best way forward. Many national leaders offered testimony on a broad range of questions and issues that the OSHA scientists and administration had proposed. The concept of dose and tumor latency as described in the paper by Jones and Grendon [55] was presented, debated, and eventually dismissed by OSHA. It was highly unfortunate that the lead author, Hardin Jones, unexpectedly died on February 16, 1978, prior to the hearings. As a consequence, he could present neither his personal views, perspectives, or understandings, nor could he clarify any questions, nor debate and defend any criticisms. For whatever reasons, the co-author, Alexander Grendon, also did not participate in the hearings. However, in a later oral history at the University of California, Berkeley [50], he acknowledged both the criticisms and his intention to address and answer such criticisms, but there is no evidence that he ever did. Several scientists from industry offered testimony at the OSHA hearings and stated their support for the perspective offered by Jones and Grendon [55]. Nevertheless, they did not, or perhaps could not, address any of the criticisms. Furthermore, other toxicologists or radiation health scientists who supported the dose-latency concept, such as Hermann Druckrey, Robley Evans (MIT) or several Russian toxicologists, also did not participate in the OSHA hearings.

On the one hand, the advocates of the dose-latency concept developed by Jones and Grendon [55] either did not attend the hearings (including Jones and Grendon) or were not sufficiently knowledgeable to explain and defend data in support of the concept. On the other hand, multiple notable critics from the fields of biostatistics and epidemiology (but strangely not from toxicology) did attend and were well prepared to present their arguments against the concept. The critics were all prominent scientists and included the pathologist Umberto Saffioti, NCI; biostatisticians Marvin Schneiderman, NCI and David Hoel, NIEHS; as well as epidemiologist Richard Peto, Oxford University; and occupational physician/epidemiologist Irving Selikoff, Mount Sinai. Without the presence of the key advocates and authors of the originating paper (i.e., Jones and Grendon) to offer a deeply knowledgeable, vigorous, and rigorous explanation and defense of the dose-latency concept, its quick dismissal is not too surprising since OSHA was left with little choice but to abandon its consideration of the dose-latency concept.

The criticisms by Hoel at the hearings, which were based on his co-authored paper [51], were that the findings of Jones and Grendon were predictably based on a stochastic assessment of the dose-response data and then implemented with contemporary mechanistic models of carcinogenesis. Hoel stated that Jones and Grendon overinterpreted otherwise predictable findings and that their analysis was neither novel nor insightful. Guess and Hoel [51] argued that the Jones and Grendon findings allow one to conclude that "on average animals exposed to very low doses of carcinogens will die of something else before developing tumors" (page 280 [51]). They argued that population risk could not be determined by the time-to-first-tumor or the extent that the time-to-tumor exceeds the average lifespan. One needed to know the mean time-to-tumor and the distribution around the mean in order to model the population response required for risk assessment. Guess and Hoel [51] presented the results of such a modeling exercise, using a linearized multi-stage model that was modified to include a time-to-tumor parameter. They determined that the 1/3 power-dependence law of Druckrey for the mean time-to-tumor or first time-to-tumor can be explained by the multistage model of carcinogenesis, assuming three stages. Use of this model leads to a Weibull distribution for the time-to-tumor. For example, the risk of one death per million occurred with the estimate that the average time-to-tumor would exceed the normal lifespan by 10-fold. The Jones and Grendon approach is simply another way of describing the population-based response, but the numerical meaning was the same.

Several key assumptions were employed in the Guess and Hoel [51] assessment: the induction time (i.e., time-to-tumor or latency period) is stochastically independent of the initiation time and functionally independent of dose. What do these assumptions mean? They indicate that initiation (i.e., mutagenic event) is a stochastic/probabilistic process and occurs independently of a second stochastic process that is the induction time-to-tumor or tumor latency, which also involves the processes of tumor promotion and progression. Although the initiation process has long been viewed by many as stochastic in nature, this has not been the case for tumor latency. Guess and Hoel [51] did not provide documentation to support their stochastic assumptions about tumor latency. The model of Guess and Hoel assumes that the response rate is linear. Thus, Guess and Hoel [51] assume tumor latency (tumor promotion) to be a stochastic event that is linear at all doses when, in fact, it appears to be both a non-stochastic and nonlinear (biphasic) even [92,93].

Guess and Hoel [51] estimated that the risk of tumor development could be appreciable during a normal lifespan, assuming no competing causes of death. However, they also showed that the risk of developing such theoretical tumors could likely decrease by a factor of 10,000 if the

ratio of mean time-to-tumor to the length of a normal lifespan (i.e., latency/lifespan ratio) increased from 1 to 20. This is a striking decrease in risk estimation with profound public health implications, even assuming a linear dose response. A more modest increase in the latency/lifespan ratio from 1 to 5 still decreases the risk by a factor of 100.

Less significant were the criticisms of pro-OSHA expert Schneiderman [86], who mainly extended the criticism of Hoel and Guess in both his testimony and his article in the New York Academy of Sciences [87]. In a technical and broadly conceptual manner, he also criticized the concept of estimating tumors that may occur after death from other non-cancer causes. Schniederman et al. [87] criticized the Druckrey concept by noting that heavy smokers developed their lung cancers no sooner than non-smokers who developed lung cancer. Similar criticisms were given that related to the median age of death from lung cancer not being related to the number of cigarettes smoked per day. These criticisms had severe biological limitations in that the types of lung tumors among smokers and non-smokers can be biologically diverse and be influenced by unique genetic factors and varied environmental exposures other than cigarette smoke. In a similar fashion, the simple relationship of age at death to the number of cigarettes smoked per day lacks important information on duration of smoking, genetic risk biomarkers, and other modifying factors, making these criticisms conjectural. The principal argument revolved around the limited capacity of epidemiological assessment to detect the practical threshold of Druckrey, especially when incidence rates are as low as they are in humans. Selikoff [89] failed to directly address the Druckrey proposal, rather noting the limitations of animal data to properly capture the human condition as based on his experience with asbestos. Thus, Selikoff was unwilling to adopt a Druckrey perspective unless it was strongly supported by epidemiological evidence, a perspective not unlike that of Schneiderman et al. [87].

A counter argument not presented to the critics at the OSHA hearings was that the relationship between dose and time-to-tumor is an important and real concept and there is no reason to restrict the assessment only to tumor incidence from the animal bioassay. In fact, Albert and colleagues [2,3,102] argued for the use of a novel approach in extrapolating animal results to human environmental health standards. Their approach incorporated the concept of age at the time of tumor appearance as well as tumor incidence, a position similar to that of Jones. They stated that the usual approach for assessing carcinogenic risks, i.e., providing the dose-to-incidence relationship, could be misleading in addition to receiving an inappropriate degree of importance in the risk assessment and risk management processes. The typical dose-incidence methodology used by regulatory agencies today fails to consider the age at which new tumors occur or the likelihood that additional carcinogen exposures could affect people who would have developed tumors from different causes. Supporting this perspective was a National Academy of Sciences (NAS) (1975) report [65] that commented upon the work of Albert and Altshuler [2]. It suggested that standards may be established for carcinogens that would limit the occurrence of environmentally induced tumors only to very advanced age, with less than a 10% increase in the chance of cancer occurring at $\geq$ 95 years of age. Although the age of 95 may not seem as old today in the third decade of the 21st century, it did seem old some four decades ago. The basic concept of the NAS was also in agreement with the perspectives offered by Jones and Grendon [55]. Again, the major OSHA carcinogen hearings failed to attract the kind of novel thinking as represented by the NAS. Likewise, Albert and Altshuler [3], both prominent toxicologists, did not render testimony at the OSHA Hearings on this issue. In the case of Albert, it was a curious omission as he was both a professor at New York University and the first head of the newly created EPA Carcinogen Assessment Group (CAG). Subsequently, Albert would support the dose-latency concept for cancer risk assessment for over two-decades, relying heavily on the work of Blum, Druckrey, and Jones and Grendon [3].

For all practical purposes, the possibility that dose and tumor latency would be adopted as a regulatory risk-based approach came to an end during the OSHA hearings. In the intervening four decades of profound debate on many aspects of carcinogen risk assessment, especially by intellectual leaders with an industrial perspective, the debate over dose-latency has itself been remarkably latent.

5. Rethinking the dose-response concept post OSHA hearings

In the intervening years, several lines of research have emerged that affect the perspectives of Druckrey and Jones. They relate specifically to the doses of carcinogens that enhance the initiation and promotion events of tumorigenesis. The following sections address each of these more recent lines of research.

5.1. The multistage model for cancer risk assessment: emerging limitations

The 1976 paper by Crump et al. [34] established the multistage model in cancer risk assessment and posited two basic assumptions upon which the entire set of cancer risk estimates rests. The first assumption is that carcinogen-induced cancers are “additive to background” and act via identical mechanisms. The second assumption is that chemical- and radiation-induced cancers are initiated in a single cell and that the tumor growth continues via clonal expansion of this altered/transformed cell. During the past decade, substantial research has exposed serious limitations and flaws in these two assumptions, showing that neither can be generally accepted and used to support LNT-based cancer risk assessment. For example, Calabrese [24] reported that an assessment of 45 carcinogens in 13 mammalian species demonstrated that all carcinogens induced the same type of organ-specific tumors as the control group but via different mutational pathways. If tumors were developed in the same organ by different pathways/mechanisms, then it seems that control and treatment tumors are also developed differently and therefore cannot simply be “additive to background” tumors. Instead of “additive to background” it may be more accurate to refer to them as “in addition to background”. The Calabrese [24] findings demonstrated that the original assumption of Crump et al. [34] lacks scientific validity. The Crump et al. [34] assumption seemed appropriate at the time since it was believed then that carcinogens were mutagens and simply worked via the same mutagenic processes. However, with advances in the understanding of oncogenes and tumor mutation sequences, it has become clear that mutagenic and carcinogenic processes are not equivalent.

With respect to the second assumption, Parsons [70,71] showed that numerous tumor types have multiple clonal origins, indicating that the original assumption of the clonal expansion of a single transformed cell has many exceptions. On balance, the “additive to background” criticism should be even stronger since no exception was found in support of the “additive to background” concept in this cited review of 45 carcinogens [24]. These newly emerging developments have exposed critical limitations of the multi-stage LNT model proposed by Crump et al. [34] and, in so doing, have discredited it as a capstone to the regulatory principles that are involved in assessing cancer risks at low doses. In fact, the criticisms by Guess and Hoel [51] of the Jones and Grendon [55] risk estimates were also predicated on the now retrospectively faulty assumptions set forth by Crump et al. [34].

5.2. Single high-vs chronic low-dose exposures: initiation, promotion, and arguments for cancer thresholds

In multiple papers, Raabe [75,76,78,79] indicated that the LNT model as applied to the atomic bomb studies is based on a faulty premise, that is, cancer incidence is due entirely to stochastic processes. Atomic bomb detonations are brief radiation exposures to blast, heat, and other trauma that occur at very high dose rates (i.e., delivered within approximately 1 min) and may act to initiate and/or promote tumor growth. Thus, individuals would have been exposed to both initiating and promoting stimuli within a similar, overlapping, and relatively short timeframe, not unlike the single, high-dose exposures in

the carcinogenic studies of Druckrey. According to Raabe [77–79], the atomic bomb detonations accelerated the development of clinically detectable tumors. Since tumor promotion is a non-stochastic and essential process of tumorigenesis, as discussed, it will require a promotion threshold to be exceeded. Epidemiological evidence of tumor incidence from atomic bomb survivors supports the argument of Raabe for solid tumors, since the tumor increase was associated with dose and closely followed a consistent lifetime pattern, independent of age at exposure.

Extensive epidemiological studies were conducted on radium-dial painters using a range of statistical models [40,42] and these studies demonstrated that the threshold and not the linear dose-response model accounted for the bone cancers induced by radium 226 [41]. Further evaluation of these data by Raabe [77] demonstrated that the radium-induced bone cancers were best represented as a function of the average lifetime "dose rate" to target tissue rather than cumulative dose. These data indicate that cancer latency at low dose rates appeared to exceed the normal lifespan, much like that reported by Druckrey, Blum, and others.

The striking findings of Evans [42] encouraged the development of a substantial series of lifetime dog studies by Raabe [77–79] using radionuclide emitters composed of both low "linear energy transfer" (LET) beta radiation and high LET alpha radiation. Consistent with the human studies of Evans [42], the dog studies of Raabe revealed that tumor latency in radiosensitive skeletal tissue was a non-linear function of the average "dose rate" from a lifetime of radiation exposures. Furthermore, the cumulative lifetime risk (incidence) for developing tumors from exposures at the threshold range actually was found to be significantly less than the lifetime tumor incidence in control animals, indicative of an hormetic response. As did Evans in his study [77–79], Raabe concluded that the bone cancers induced by threshold doses had latency periods that were longer than the normal lifespans of the animals. He also concluded that these results from animals with internalized radionuclides would apply to all forms of protracted exposures to ionizing radiation, including from external sources. These repeated results would have widespread application to drinking water standards, food regulations, and soil remediation standards, all of which currently rely on the assumption of LNT carcinogenesis.

In accord with the differential relationships of carcinogenic doses to initiation and promotion, a substantial database of single-exposure carcinogens was developed that included some 6000 experiments [28]. It was of particular relevance that a substantial tumor-promoting presence was required for single-exposure carcinogens to induce cancer, either because of the treatment process itself, as in high dose carcinogenic studies, or because of the endogenous presence of strong tumor-promoting stimuli, as exemplified below by the Sprague-Dawley female mammary gland on days 48–52 of post-natal life [55].

Jones and Grendon [55] integrated many high-dose, single-exposure, carcinogen studies that were adopted from the original research of Druckrey and, as such, provided many dose-dependent examples of mutational events and tumor-promoting activities. It was the tumor-promoting aspect of carcinogenesis, however, that was thought to be the dominant feature in affecting tumor latency—time between initiation and tumor development. One classic example (i.e., out of hundreds of illustrative examples in toxicology) of the effect of promotion on tumor latency is that of a single dose of DMBA. When administered to Sprague-Dawley female rats on day 50 of adult life, DMBA resulted in the occurrence of mammary tumors within only several weeks. If the same dose were administered just one week earlier or later, however, the tumor formation would be profoundly diminished and delayed. The difference between these respective tumor latencies is due largely to the temporal difference in the innate tumor-promoting potential of the target tissue. That is, between 48 and 52 days of age, the female Sprague-Dawley rat undergoes an expansive proliferation of mammary tissue. It is this burst in developmental activity between days 48 and 52 relative to its absence prior to day 48 and after day 52 that accounts for the heightened endogenous tumor-promoting activity on DMBA-initiated cells and, ultimately, for most of the DMBA-induced formation of mammary tumors [55].

The idea that chemically induced tumor promotion may occur in the chronic bioassay has long been recognized. Ames emphasized this issue when he addressed the possible concerns inherent in using data from animal bioassays to extrapolate to and predict cancer risk in humans. In particular, Ames et al. [4] discussed the likely potential for high doses of toxic chemicals to induce a range of reparative responses in tissues, including DNA repair/synthesis and cell proliferation. Ames and others have indicated that such proliferative responses to highly toxic doses challenge the validity of chronic bioassays—especially when coupled with LNT modeling—to be useful tools for accurately predicting risk at low doses. Although the toxicity-induced proliferation occurring at the high doses used in bioassays is of legitimate concern for overestimating risks at relatively low, non-toxic doses, a related point that generally escapes attention is that the tumor-promoting activity of high toxic doses not only enhances the observed tumor incidence but may also shorten the tumor latency by accelerating tumor development. Although considerable data support the inverse relationship between carcinogen dose and tumor latency, including responses from the massive ED01 study [16], the chronic bioassay would not be able to assess this relationship because the interim sacrificing of animals in this assay is typically precluded.

Several other important dose-latency issues merit consideration. Although Druckrey and others addressed the issue of tumor promotion across the entire dose-response continuum, they failed to consider the situation in which a low dose has no demonstrable impact on tumor promotion. Considerable data demonstrate that initiated/transformed cells can remain for a large fraction of an animal's lifespan in an unpromoted, precancerous, and pre-tumorous resting state. With the administration of an exogenous tumor promoter, the process of carcinogenesis may be activated and accelerated. It is this type of delayed activation about which Jones and Grendon have written. That is, a biological condition can exist whereby a tumor does not develop during one's normal lifespan due to the lack of adequate tumor-promoting activities or their proper timing. The possibility also exists that tumor-promoting activity is present but weak. If this is the case, then one might expect progress toward tumor formation but with a significantly prolonged time period. In such circumstances the dose-latency relationship would be non-linear as would the dose-response relationship.

The dose response for DMN-induced kidney cancer in rats [35] showed a linear dose response for DNA adduct and proliferative foci formation, while displaying a threshold-like response for tumor incidence, suggesting a multi-stage process which includes the disappearance of most early foci. In this context, models have evolved to incorporate mechanistic developments and tumor-promoting activities. In such models, spontaneous pre-cancerous cells are often assumed to be present and independent of the carcinogen treatments. A subsequent tumor-promoting treatment may then enhance the rate of conversion of spontaneous and carcinogen-induced precancerous cells to malignant cells via non-stochastic processes. This concept may be applied to chemical- and radiation-induced carcinogenesis [53,90,91].

5.3. Thresholds for tumor promotion

Several toxicological developments have supported a reappraisal of the dose-latency relationship. In the mid-1980s, Slaga [92,93] and colleagues established a causal relationship between dose, mutations, tumor promotion, and latency. They reported that while low doses of some PAH carcinogens initiate the process of carcinogenesis, higher doses of the same agent not only initiate but also activate a plethora of tumor-promoting activities. Such higher doses accelerate carcinogenesis, resulting in significantly shorter latency periods. Slaga and co-workers never related their findings back to Jones and Grendon [55], Guess and Hoel (1977), or the OSHA carcinogen hearings (1980).

However, their findings provide valuable insights because activation of a broad spectrum of tumor-promoting processes challenges the stochastic approach of estimating cancer risks based on mechanistic events at low doses. The Slaga findings also suggested a mechanistic basis for non-linear dose-response relationships, as most low doses of carcinogens were unlikely to induce significant or even quantifiable tumor-promoting activities.

Furthermore, in the four decades since publication of the critical comments of Guess and Hoel [51] and the OHSA Hearings [67], no clear restatement of the proposal of Jones and Grendon has been enunciated despite many advances in the understanding of the mechanisms of chemical- and radiation-induced cancers. Nonetheless, demonstrable evidence now exists indicating that dose can affect both the initiation and promotion processes of carcinogenesis [81]. For carcinogens then, the magnitude of the dose may influence initiation and promotion as well as progression. The lower the dose, the lower the potential for a chemical carcinogen to transform a tumor into a cancerous (i.e., malignant) state [81]. Exposure to lower doses of a carcinogen was reported to result in the development of tumors that were dependent on promotion, whereas exposure to higher doses of the same carcinogen resulted in the development of tumors that were independent of promotion. Thus, if the tumor-promoting activities were removed from animals given low doses of carcinogens then the tumor growth would recede in the absence of the tumor-promoting agents. This would not happen, however, in animals receiving higher doses of a carcinogen. These findings are consistent with studies reporting that high doses of initiators can cause carcinomas even in the absence of exogenous tumor promoters [28,81,93,96]. In fact, the capacity for a sufficiently large single dose of a carcinogen to induce cancer in an animal model has been commonly reported in the literature, yet this fact apparently is not widely appreciated.

These findings indicate not only that the latency period is an inverse function of the dose of a carcinogen but also that this dose-latency response is not a stochastic process. Initiation by a single low dose of carcinogen and promotion by a tumor promoter result in tumors more quickly than initiation alone in the absence of tumor promotion. Thus, such findings of Reddy and Fialkow [81] provide a foundation that can account for thresholds occurring as a result of the dose-response relationships for carcinogens.

The data indicate that not only is dose a significant factor in affecting the time-to-tumor, but when linked to a dose that does not significantly affect tumor promotion then the most likely response is a threshold response. The integration of these two concepts results in a biologically based process that makes a threshold response the most toxicologically dominant and preferred model for estimating safe (subthreshold) responses to chemical carcinogens and ionizing radiation.

5.4. Nrf2-mediated hormesis: mechanistic basis for carcinogen-induced thresholds

It is widely accepted that inflammation is a significant driver of carcinogenesis, especially of tumor promotion [73]. Within this context, it is important to emphasize that a very common hormetic mechanism involves the activation of Nrf2, a ubiquitous and abundant transcription factor with potent anti-carcinogenic potential. Briefly, hormetic stimuli trigger mild bursts of reactive oxygen species (ROS) that activate and then mediate the migration of Nrf2 from the cytoplasm to the nucleus. Once in the nucleus, Nrf2 upregulates the expression of a vast complex of genes that mediate many downstream responses involved in regulating both the inflammatory and redox status of cells. Of significance here is that most of these downstream responses actively suppress the initiating and tumor-promoting aspects of many carcinogens [29,56,62,69]: an increase in the repair of carcinogen-damaged genes and proteins; an enhancement in the detoxification and clearance of carcinogens; an upregulation in the expression of the antioxidants and antioxidant enzymes that neutralize both the initiating and promoting effects of oxy free radicals; and the downregulation of the proinflammatory transcription factor NF-kB as well as a reduction in the cytokine-mediated inflammation that is thought to exacerbate the process of tumor promotion.

It is important to note that these Nrf2-mediated anti-carcinogenic responses require a mild burst of ROS and that this ROS burst itself is an integral and apparently necessary part of the hormetic response to low (i.e., hormetic) doses of stressor agents, including carcinogens. At its core, hormesis is a biphasic nonlinear dose response that exhibits a defined threshold to toxic agents. It is also an adaptive mechanism that enhances resiliency in response to subthreshold doses of toxic agents and thus protects cells from ever-increasing levels of acute and chronic stressors. On the other hand, Nrf2 and its activation represents at least one apparent basic molecular mechanism by which hormesis mediates many of its antioxidant, anti-inflammatory, and anti-carcinogenic responses.

Numerous types of hormetic exposures to various specific agents (physical, chemical and biological) or various conditions and behaviors may activate Nrf2. These include exercise, mild heat stress, intermittent fasting, low-level light, ionizing radiation, a broad spectrum of environmental contaminants (such as arsenic, benzene, cadmium, fluoride, hypochlorous acid, lead, methylmercury, zinc, Bisphenol A, and PFAS) as well as a vast spectrum of phytochemicals (such as fish oils, curcumin, green tea and some of its constituents, such as EGCG, ginkgo biloba, ginseng, sulforaphane from broccoli, and resveratrol from red grapes) [15,69,106]. Numerous pharmacological studies have demonstrated that an array of agents and processes were therapeutically effective and afforded protection to the brain, heart, liver, kidney, skin, and other organs. In all cases, their therapeutic effectiveness was related to the activation of Nrf2 [57]. Also, tissue protections induced by an assortment of preconditioning protocols are now considered largely mediated via the activation of Nrf2 [48]. As alluded to earlier, Nrf2 is part of an interconnected network of transcription factors that participate in biological crosstalk and whose regulatory influences on each other's activities are based on dose-response and temporal relationships, all functioning within the biological context of optimizing performance [49].

Numerous toxicological studies have shown that activating Nrf2 with low doses of a variety of chemical and physical agents blocks the onset of toxicity and increases the threshold for subsequent toxic exposures [59,105]. Studies with Nrf2 knockout mice support such findings by demonstrating that the reverse is also true, that is, the absence of Nrf2 markedly enhanced the onset and severity of both the toxicities and disease processes induced by low doses. Essentially, low doses of Nrf2-activating agents completely prevented the toxic effects and dampened the inflammatory responses to below that of control groups [52,61,98] by an apparent mechanism that is consistent with a hormetic-like J-shaped dose response [15]. Notably, these examples indicate that activation of Nrf2 is an hormetic response that can diminish the occurrence of genetic damage as well as inflammation.

The activation of Nrf2 provides not only a possible mechanistic basis for a threshold response [5] but also a method for reducing tumor incidence below background and extending latency beyond that of controls, that is, a beneficial Nrf2-mediated hormetic response [29]. A striking example of such a reduced tumor response may be the hormetic J-shaped response observed for DDT-induced liver cancer in the F344 rat [47], a beneficial response that now may be linked to the activation of Nrf2 [63]. Furthermore, modest caloric restriction (CR) that acts via Nrf2 activation [64] significantly reduced the incidence and extended latency of radiation-induced myeloid leukemia (as once appeared in Nagasaki and Hiroshima) in C3h/He mice [103,104]. The latency of onset for first cancer was 468 days for mice undergoing CR after being irradiated (3 Gy) as compared to a latency of only 330 days for irradiated non-CR mice, extending latency 138 days for CR mice. The prolongation of the latency was extended even further, that is, by 356 days, when the CR was given before the irradiation. One conclusion from this study is

that CR-activated Nrf2 likely mediates the suppression of both the initiating and promoting stages of carcinogenesis.

Mechanistic developments over the past 15 years have enlightened the dose-latency debate as presented in this paper. Essentially, the mechanistically interconnected relationship between Nrf2 and hormesis reveals the importance of low doses and low dose-mediated adaptive responses in blocking and even reversing agent-induced diseases, including cancer. The relationship between Nrf2 and hormesis predicts that low hormetic doses of carcinogens can significantly extend tumor latency, even to beyond the lifespan of untreated controls, a truly protective (beneficial) response. This extension of tumor latency is in addition to the reduction in tumor incidence, responses that are both likely mediated, at least in part, by the hormetic activation of Nrf2. The roles of hormesis and Nrf2 in both extending tumor latency and reducing tumor incidence to below control values argue strongly against LNT as the valid universal dose-response model for use in cancer risk assessment. For carcinogens, hormesis predicts a biphasic dose-response that exhibits a threshold dose and a lower-than-control tumor response to subthreshold (hormetic) doses of carcinogens.

6. Conclusion

This assessment describes and supports the existence of thresholds for chemical carcinogens and ionizing radiation. The conclusion is based on findings that refute the additive-to-background concept of tumor incidence. Tumor latency is an inverse function of dose and has an effective threshold value below which doses extend tumor latency beyond the normal median lifespan of the control animals—even under conservative LNT dose-response assumptions—thereby strongly indicating the existence of a latency threshold that could and arguably should be used in cancer risk assessment. Tumor promotion is a non-stochastic process that yields threshold responses at sub-tumor-promoting doses. In other words, the inverse relationship between a dose of a carcinogen and its tumor latency translates into a threshold response for tumor promotion. These concepts have important implications for chronic, low-dose, long-term dosing with carcinogens as well as for acute high-dose treatments, with the capacity for affecting initiation, promotion, and progression. The Nrf2-mediated hormetic (biphasic) dose response elicited by many carcinogens indicates the presence of a defined threshold dose, below which tumor latency (and thus tumor promotion) is delayed to beyond the normal lifespan, and tumor incidence is diminished below that of control values. Also, the Nrf2 response is likely to be augmented by other metabolic and physiological changes that result in tumor diminution. These could include the stimulation of genetic damage repair in the developing cell clones, enhanced anti-cell immune responses, and changes in the tumor vasculature and environment. Regardless of the type of mechanism and dosing regimens (i.e., chronic or acute), low doses yield thresholds.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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