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Review Article

Linear non-threshold (LNT) fails numerous toxicological stress tests: Implications for continued policy use

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ABSTRACT

The linear non-threshold (LNT) dose-response model has long been employed by regulatory agencies to assess cancer risks from exposures to chemical carcinogens and ionizing radiation. Herein a series of fundamental historical, physical, chemical, and biologically based toxicological "stress tests" were "administered" to the LNT model, showing important limitations for its use in low dose extrapolation for all endpoints but with particular focus on cancer risk assessment where it is commonly applied. These limitations reveal that its capacity to make low-dose cancer-risk predictions is seriously flawed, precluding its use as a reliable model to estimate low dose cancer risks.

1. Introduction

The linear non-threshold (LNT) model has been the sacrosanct dose-response model used in cancer risk assessment for over half a century. At the core of the LNT model is the belief that a single carcinogenic molecule or a single ionization can initiate the complex process of carcinogenesis, which may then be promoted via various endogenous (i.e., background) and exogenous factors into a fully developed and cancerous tumor. In spite of its overwhelming preeminence, however, many studies and assessments have been conducted during the past several decades that have questioned the credibility of some fundamental assumptions and historical foundations underpinning the scientific basis of LNT. The studies have ranged from the molecular to the population-level and have proven to be particularly persuasive among some radiation scientists and toxicologists [1–8], piquing interest in and skepticism of the LNT model. Nevertheless, despite the abundance of contradictory evidence and of legitimate skepticism, regulatory agencies continue to use LNT as a central tool for assessing carcinogenic risks and determining policies.

It is therefore of considerable importance to provide an integrative, comprehensive and critical assessment of the scientific basis of LNT. The present study provides a series of scientific "stress" tests (much like other stress tests to insure economic wellbeing and personal health) to identify the capacity and robustness of the LNT dose-response model to predict a broad range of biological endpoints, with particular emphasis on cancer risks in the low-dose zone. The broad range of challenges reveals that the LNT model has fundamental limitations, from its historical origins based on errors and misrepresentations of the scientific record to its many inconsistencies with evolutionary, molecular and organismal biology.

The first section offers an historical perspective on LNT, providing background and context to subsequent sections while enumerating many opportunities to evaluate the capacity of the LNT model to properly frame, describe and predict responses of ionizing radiation and toxic agents in the low dose zone.

2. Historical origins of LNT

2.1. Muller and the Proportionality Rule

Olson and Lewis [9] first proposed the LNT concept in 1928

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following the claim that X-rays could produce copious gene mutations in the germ cells of fruit flies [10]. They assumed an LNT dose response based on these studies to provide their explanatory mechanism of evolution. Two years later, Muller [11] (see Hanson [12]) proposed the concept of a dose-response Proportionality Rule for induced gene mutation down to a single ionization particle.[1] This belief was based on the extraordinary extrapolation over about 25-million-fold (dose rate) exposure to background radiation exposures under the assumption that all exposures, no matter how small, caused gene mutation and such damage was not reparable [13]. This dose response model was given a mechanistic explanation five years later when Timofeeff-Ressovsky et al. [14] linked target theory of physics to the mutation data of Muller and colleagues, creating the LNT single-hit model with its capacity to estimate responses in the low dose zone. However, the target theory single hit LNT model of Timofeef-Ressovsky et al. [14] likewise included no provision for gene mutation repair [17].

A fundamental assumption of the LNT model was that Muller had induced gene mutation. However, this would prove to be a highly problematic point of scientific contention. While Muller would claim that he induced tiny "point mutations" within individual genes this was found to be untenable over the next two decades by a diverse group of radiation geneticists [18–20]. Muller's attempt to support his gene mutation claim via the study of "reverse" mutations was repeatedly discredited (see Lefevre [18]), with Muller [21] eventually acknowledging that his high dose exposures produced their transgenerational phenotypic changes via the induction of changes at the chromosomal level principally via modest to massive gene deletions.[2] Later studies with nucleotide measurement technologies affirmed this conclusion that Muller's groundbreaking research that produced gene mutations was an incorrect interpretation of his findings [22, 23]; see Calabrese [34], page 9, left column for numerous other supportive references). As Stadler [20] aptly noted, Muller had confused an observation with a mechanism. Thus, the LNT single-hit model for carcinogenesis was wrong from the start, being based on an incorrect assumption of gene mutation, yet it was accepted as accurate for decades, strongly influencing cancer risk assessment principles and practices at regulatory agencies such as EPA in the 1970s to the present.

The LNT concept struggled for broad acceptance during the intervening years after Muller [11] proposed the Proportionality Rule [25, 26], especially within the medical community that supported a threshold dose-response model. However, it received a powerful boost when Muller received the Nobel Prize in 1946 with his claim during his Nobel Prize lecture that the threshold model should no longer be used for radiation risk assessment, basing his comments largely upon the dissertation research of Ray-Chaudhuri [27] whom he directed at the University of Edinburgh[3] from 1938 to 1939. Muller [28] would state that the findings of Ray-Chaudhuri "leave, we believe, no escape from the conclusion that there is no threshold dose …." The Ray-Chaudhuri dissertation had numerous concerns including the piloting of the study with different insect strains/crosses than used during the study, the changing of the insect model midway during the study due to the fact that the originally selected strain cross could not be successfully used to answer key research questions (i.e., assessing mutation and translocation in the model at the same time), the combining of data across different strains, and other documented concerns. It becomes evident that this dissertation did not anticipate a series of research problems and was constrained by serious time concerns due to the start of World War II that pressured Muller to return to the US and Ray-Chaudhuri to India. Thus, the lack of adequate preliminary testing, the occurrence of necessary midcourse changes that could not be piloted and imposed time constraints markedly affecting the quality of the dissertation and scientific value.

Another scientific issue with the Ray-Chaudhuri dissertation is that it did not indicate whether the control and the radiation treatment group were in separate incubators in the same room and adjacent to each other as was the case with the Caspari and Uphoff studies at the University of Rochester during the Manhattan Project where Muller was a paid consultant. Of importance is that the gamma rays from radium had the potential to affect the nearby controls. To mitigate this concern, a lead shield was placed between the two incubators. Despite this effort to block the radiation, the controls were exposed to about 1% of what the treatment group received based, resulting in an additional total dose of about 0.6 r, with a dose rate that was about 100 fold greater than background. Thus, the control groups of the Caspari and Uphoff studies reflected an exposure rate approximately two orders of magnitude in excess of background with no apparent increase in the control mutation rate. In the Ray-Chaudhuri study there was no information provided concerning where the control and treatments were located or about lead shielding. If the control and treatment groups were similarly handled as with the Caspari and Uphoff controls, the estimated exposure would be about 24 r over the 30 day study. Given that the control did not show evidence of excess mutation, two options are most probable: the controls were moved sufficiently from the radium source to make exposures so low as to be inconsequential or that the groups were close enough to

[1] The Proportionality Rule, as formulated by Muller [11], was based on his view that the genome of the fruit fly was very protected and stable as it had shown only 400 visible mutations in about 20 million flies, even after massive testing with highly toxic agents trying to induce mutations [15]. Muller failed to consider the possibility that the observed very few visible mutations could have occurred via other circumstances such as with a susceptible genome but with efficient repair as is now known to be the case [16]. However, with this second option there would have been different dose response implications suggesting the possibility of threshold or hormetic dose response models. Thus, Muller's limited perspective was not founded well with respect to evolutionary biology. In fact, if Muller had been more open, creative, visionary or perhaps less biased then he would not have proclaimed a "Rule" but an "hypothesis" rather than a poorly supported Rule which has come to improperly dominate the scientific and regulatory communities to the present.

[2] In 1956, Muller [21] acknowledged that collective research with Drosophila indicated that a substantial proportion of what he originally referred to as "point mutations" were now seen as gross genetic deficiencies/deletions and other structural chromosomal changes, supporting the long-standing position of Lewis J. Stadler. Muller [21] wrote that "there is no doubt that in X-rayed Drosophila also, at least when the irradiation is applied to condensed chromosome stages, such as those of spermatozoa, deficiencies as well as other demonstrable structural changes arise with much higher frequency relative to changes that appear to involve but one gene …." The statement must have been difficult for Muller to write since it eroded his long-time position asserting the primacy of "point" mutations. In essence, even though it took 25 years, Stadler had won the dispute.

[3] A detailed assessment of the Ray-Chaudhuri dissertation along with a series of letters between Muller and Ray-Chaudhuri revealed a large number of inadequacies and flaws which seriously compromise its scientific value [29]. In addition, Ray-Chaudhuri and Muller omitted (i.e. hid) essential methodological limitations and non-supportive findings. Using this series of deceptions, Muller challenged the continued use of the threshold model based upon the Ray-Chaudhuri dissertation. He made this assertion in no less of a setting than his Nobel Prize lecture [29]. Muller would continue to cite the Ray-Chaudhuri study during the BEAR I Panel discussions arguing that the total dose hypothesis held over a 120,000 dose range (Muller letter to Weaver, January 21, 1956 [30]. No contemporaries would challenge these "authoritative" assertions. It was due to an evaluation of the personal Correspondence and related documents that the many research irregularities and deceptive reporting were uncovered [29].

require lead shielding. If the controls were exposed to an additional 24 r over the 30 days, then the findings would indicate there was no obvious treatment effect at now 4000-fold dose rate greater than background, suggesting a threshold response. However, failure to report this information makes the Ray-Chaudhuri data uninterpretable with respect to matters relating to the issue of threshold. Despite this fundamental limitation, Muller [28] used this dissertation, as noted above, to dismiss the continued use of the threshold model during his Nobel Prize Lecture. This discovery discredits Muller's threshold dismissing proclamation during his Nobel Prize Lecture.

The Ray-Chaudhuri [27] research was designed to overcome the growing concerns over Muller's assertions that he had induced gene mutation. This dissertation tested whether induced mutations were best explained by total dose or dose rate. Muller supported the total dose or "piggy bank" hypothesis wherein genetic damage was assumed to be cumulative, irreversible and irreparable leading to a linear dose response relationship. The dose rate hypothesis suggested the presence of repair mechanisms, along with the possibility of a threshold dose response. Of particular significance is that due to Muller's influence the total dose vs dose rate test would be become the central question that the Manhattan Project would attempt to answer. The resolution of this question was of critical importance since an affirmative answer could be used to support Muller's gene mutation and LNT assertions.

2.2. The Manhattan Project

Once genetic mutation studies were to be undertaken at the University of Rochester during the US World War II Manhattan Project, Curt Stern added Muller as a consultant in 1943. Muller convinced Stern to replicate the Ray-Chaudhuri dissertation research but in a far bigger and better manner, with the intention of overcoming many of its limitations. While the Stern-Manhattan Project research has been assessed in considerable detail [31], the major chronic exposure study component by Ernst Caspari displayed a threshold dose response for mutation with Drosophila,[4] creating a major concern for the Stern-Muller team who were ardent LNT proponents. After a number of failed attempts to find flaws in the Caspari study, mostly centered on incorrectly claiming that his control group was aberrantly high [33,34], Stern obtained funding to replicate this study with a new graduate student, Delta Uphoff. Uphoff conducted three experiments with each about one half the size of the Caspari study. Problems were encountered in the conduct of the Uphoff studies, starting with experiment #1, which was deemed as uninterpretable by the authors themselves due to an aberrantly low control group [35]. Similar aberrantly low control group values also occurred in a follow up experiment, likewise affecting its credibility. Uphoff and Stern [36] would publish a one-page summary of their three experiments along with data from the earlier two experiments of Warren Spencer (i.e., acute exposure study) [37] and Caspari (i.e., chronic exposure study) [38]. A major additional problem of the Uphoff studies is that none were ever peer reviewed and the data from the final two experiments, which are the critical chronic studies, were never seen and have been missing for 70 years. Uphoff and Stern [36] promised to provide the details of the three Uphoff experiments in a subsequent paper but failed to do so. Nonetheless, the scientific community, including their peers in the area of radiation genetics, continued to cite the one-page non-peer reviewed summary, which became a cornerstone of support for LNT.[5]

After Robley Evans [41], a prominent health physicist at MIT, provided support for the Caspari findings of a threshold and engaged other radiation geneticists on the possibility of a threshold response for ionizing radiation and mutation [42], Muller became concerned that the LNT model may lose support and be in trouble. Muller then published three papers that directly contradicted his previous letters to Stern that had strongly supported the reliability of the Caspari control group while being critical of the aberrantly low Uphoff control data. Muller published these articles to blunt the possible impact of the Caspari findings [43–45]. The documentation of Muller's written opinions was revealed in letter correspondence between Stern and himself. It is possible that Muller may have believed that this information never would have been discovered, revealing his striking inconsistencies and possible dishonesties (See Calabrese 2013 [33] for the series of Muller and Stern correspondence).

2.3. Biological effects of atomic radiation (BEAR) I Genetics Panel: recommends LNT

A major risk assessment policy change occurred in 1956 when a Muller led US NAS/NRC BEAR I Genetics Panel[6] recommended a switch from the threshold to the LNT model, based heavily on the problematic and poorly documented Uphoff chronic study data of the Stern-Manhattan Project. A recommendation was soon generalized to somatic cells for cancer risk assessment by the National Committee for Radiation Protection (NCRP) (December 1958) [46,47] and subsequently adopted by leading national/international advisory committees and governmental agencies [48]. The highly influential LNT recommendation issued by NCRPM for germ mutation and cancer endpoints was based on a precautionary principle and the idea that mutations were cumulative, non-repairable and irreversible [47] (see Calabrese 2021 [49,50]).

A profound bias in the actions of the BEAR I Genetics Panel toward the research of James V. Néel affected their risk assessment recommendations to the country. Néel was the director of the genetics studies of the atomic bombings in Japan and a member Genetics Panel. A ten year study of over 75,000 offspring of parents exposed to the radiation from the bombings revealed no treatment related effects [51]. Néel offered to share with the BEAR I Genetics Panel the major study for their review at the first meeting of the Panel. Transcripts reveal that Muller

[4] On January 14, 1947 [32] Muller sent Stern a letter evaluation of the Caspari research report showing these threshold findings. Muller provided an in-depth review on this research, concluding that he had little to find fault with, high praise from the recent Nobel Prize recipient. Muller's principal comment was that Stern needed to replicate the critical Caspari findings.

[5] It remains uncertain and speculative why Uphoff and Stern failed to publish detailed methods and results of the three key Uphoff experiments. Recent discoveries of Stern letters indicate that two of the three experiments were shortened, possibly contributing to their reduced sample size (i.e.~50%) compared to the Caspari study. There was also concern that the Uphoff experiment(s) had been affected ("i.e. contaminated") by a new substrain obtained from Muller (Stern letter to Muller [39]). Likewise, there were methodological issues raised concerning how diets were made up in the first two experiments with differences between treatment and control groups (Novitski letter to Stern [40]). There is no evidence concerning how these issues were addressed or resolved. Nonetheless, these multiple issues suggest that there may have been concerns that lead to the failure to publish the findings. In any case, these developments raise new questions with these experiments, along with the aberrantly low control mutation values for two experiments. Furthermore, failing to publish these findings is curious especially since Stern had become Editor-in-Chief at *Genetics* where he published the detailed Spencer and Caspari papers. Yet, publication of the detailed Uphoff findings never occurred.

[6] BEAR I Panel refers to the Panel that was created in November 1955 and issued their report on June 12, 1956. The Panel remained active until 1964 with a series of chairs. The remaining BEAR Panels are not distinguished by separate numbers.

lead the Panel to reject giving the study scientific standing and it was not assessed,[7] leaving the Panel to rely upon the non-peer reviewed and unpublished data of the Uphoff experiments. Néel would give his study to a similar British genetics/human population committee where it was influential.[8] These interactions would later result in extremely strained relationships between Néel and Muller, fracturing relationships within the closely knit radiation genetics community [51].

The BEAR I Genetics Panel geneticists were asked to provide estimates of radiation –induced genetic damage/birth defects in the first and tenth generations after a parental exposure to 10 rads. The "best" estimates by the panelists for generation #1 ranged from 2 to 10 million offspring based on the size of the 1956 US population. However, the 10 year study of Néel showed no treatment effects after following 75,000 offspring over an even higher dose range (up to 150 rads) than assumed by the panel. Thus, the Néel data directly contradicted the predictions of the Panelists. Nonetheless, the BEAR I Genetics Panelists refused to incorporate as well as share the Néel human population study data, choosing the predictions based on fruit flies and mice over extensive human data. This decision reflected the leadership of Muller and the Panel's ideological biases. This ethically questionable strategy worked very well as their message was the only one that the public heard from the major news outlets (e.g., *New York Times, Washington Post*),[9] while the Néel study was ignored.

Another concern with the actions of the BEAR I Genetics Panel was their deliberate attempt to hide the striking uncertainty of genetic damage/birth defect estimates of the individual geneticists and the extent to which the geneticists differed between themselves. In fact, it has been shown that the 12 geneticists were asked by the Chair of the panel (Warren Weaver) to provide detailed written estimates of genetic damage independently over a one month period. Nine panelists accepted the challenge and provided their estimates within the one month period with three declining based on their belief that any such estimates would be too uncertain to be useful. To both the shock and disappointment of the Chair, the estimates of the nine geneticists were profoundly divergent, along with great uncertainty within the error bounds of individual estimates[10] [24]. This "simple challenge" created a major crisis because of its implications. It immediately suggested that any recommendations of the panel would be meaningless since they would be wrapped in profound uncertainty. In this moment of crisis panelist James Crow, who was charged with organizing the information, decided to drop the three most divergent estimates, reducing the uncertainty range by over 80%, hoping to save the panel efforts. When the panel published their report in *Science* it revealed that 12 geneticists were invited to provide damage estimates, as noted above, but only six took up the challenge and did so. This was a false statement that misrepresented the research record. It was apparently done to disguise the unacceptably high variability on damage estimates in order to protect their capacity to make acceptable LNT policy recommendations [24,52].

In 1963 the BEAR Genetics Panel (then chaired by James Crow) [56] was asked to advise the NAS on whether plans should be made for a second phase of the Néel atomic bomb transgenerational birth defects/genetic damage study, this time adding a second generation. This idea offered the potential for assessing a higher proportion of recessive gene mutations than occurred in generation #1. A Panel subcommittee (Néel, Sturtevant, and Stern) recommended against a follow up study of the second generation of children of exposed parents at Hiroshima and Nagasaki. The subcommittee concluded; "It is highly improbable, in view of the results of the studies on the children of irradiated survivors of Hiroshima and Nagasaki, that studies of the grandchildren of these some persons could be expected to result in statistically significant evidence for the genetic effects of the atomic bombs." This recommendation was significant in that it emphatically rejected the earlier Panel predictions of genetic damage for multiple generations as massive and detectable. This repudiation, in contrast to their LNT recommendation, was not shared with the scientific community nor media. In the end, Néel had won the day with his quiet persistence and the fact that human data should guide policy, when possible. However, this discussion concerned a large study, not risk assessment policy, which was still based on the animal model data.

2.4. The Lewis paper

Generalizing the linearity of the radiation dose response from germ to somatic cells was a seminal event, occurring after the NAS BEAR I Genetics Panel Reports (i.e., technical and Public) of 1956 [54,55]. Even though this Panel carried the greatest scientific credibility, the NCRPM [47] effectively promulgated the major policy change. This faulty generalization of linearity from germ cells (i.e., mature spermatozoa lacking DNA repair) to DNA repair competent somatic cells had a major impact on cancer risk assessment as it was adopted by Lewis for his underlying mechanistic foundation. The influential Lewis [57] article fueled interest and awareness in the likelihood of radiation-induced somatic mutations causing leukemia and other cancers. This major challenge to the threshold dose-response model was set within a highly charged political environment due to concerns with radioactive fallout in the US.

A reassessment of the Lewis [57] paper showed it to be scientifically flawed and highly biased. The Lewis [57] article was published in *Science*, received an editorial endorsement which was rare [58], making it very noteworthy. This recognition lead to Lewis becoming part of a major story on radiation risk in *Life* magazine and testifying to the US Congress,[11] both within one month of publication. Despite its notable influence on the adoption of LNT for cancer risk assessment, serious concerns have been raised concerning the capacity of Lewis to undertake this study based on this scientific education and training (i.e., trained in fruit fly genetics, but with no training in epidemiology, cancer biology,

[7] On page 6 of the November 20, 1955 of the BEAR I Genetics Panel transcripts Muller stated; "We should beware of reliance on illusionary conclusions from human data, such as the Hiroshima-Nagasaki data, especially when they seem to be negative."

[8] The British expert Genetics/Human Population Panel concluded: "We consider, therefore, that an individual could, without feeling undue concern about developing any of the delayed effects, accept a total dose of 200 r in his life-time, in addition to radiation from the natural background, provided that this dose is distributed over tens of years and that the maximum weekly exposure, averaged over any period of 13 weeks, does not exceed 0.3 r." (page 62, item 255 [53]).

[9] The BEAR I Genetics Panel produced two Reports, a "technical' one published in *Science* in June 1956 [54] and a "Report to the Public" that was published and released on June 12, 1956 [55]. The "Report to the Public" was sent to all libraries in the US. It is the Report to the Public that made headlines in the major media outlines such as the *New York Times* and the *Washington Post* and that led to Congressional Hearings on radiation health issues. It was recently learned that the Report to the Public was not written by the Panel but by a "third" party. Further, the Panel did not review or approve the Report to the Public prior to its publication and release. The recently discovered letters of Panelists Hollaender, Muller, Russell and Sturtevant note that the Report to the Public contains serious errors and that the Report did not represent the views of the Panel. Further, the National Academy of Sciences represented the Report as being the product of the Panel and representative of its views which would be a serious breach of ethics.

[10] Panelist George Beadle provided a range of damage estimates from a low of 100,000 to a high of 200,000,000. It was such extreme examples of uncertainty that created great concern in Crow and Weaver. If such uncertainties were revealed, the public would be unable to consider Panel recommendations seriously.

[11] Testimonies in 1957, 1959 and 1960 by Lewis to the U.S. Congress were also extremely biased. In fact, a strong case could be made that he deliberately misled Congress to support his LNT views on cancer risk assessment (see Calabrese [48]).

leukemia, radiation chemistry and dosimetry, quantitative risk assessment methods), and the scientific quality and objectivity of the publication [49,50]. For example, Lewis focused on leukemia in four groups exposed to ionizing radiation: the victims of the atomic bomb (AB) explosions, patients with ankylosing spondylitis (AS) and enlarged thymus (ET) and radiologists. In each case, Lewis failed to do the appropriate research and/or misled the reader on the status of the science. In the case of AS, the authors of the key study and its scientific oversight committee independently reported that the study should not be used for low dose cancer risk extrapolation whereas Lewis did so, failing to inform the reader about these important opposing opinions/perspectives [59]. In the case of ET, the authors of the critical study specifically stated that there was no casual relationship between the radiation exposure and leukemia in their patients, once again a view that Lewis failed to share [60,61]. The radiologist data employed by Lewis, included extremely high exposures in earlier decades, making low dose risk predictions of little/questionable value, yet these findings were masked in the Lewis paper. Lewis's interpretation was quickly discredited in a follow up study with more relevant occupational exposures [62]. With respect to the AB victims, the Lewis analysis misrepresented the dose response in the low dose range by combining exposure groups, leading to a distorted linear dose response. Numerous other analyses of the AB victim data with non-combined dose spacing have shown either threshold or J-shaped dose responses, again missed by Lewis [49,50]. Furthermore, Lewis did not consider alternative causation nor did he share such possible causal explanations as had been considered by others at that time. The apparent failure to scientifically vet the Lewis paper also calls into question the nature of the peer review process at Science.[12]

2.5. The Russell cover-up story-impact on LNT acceptance

Another example of disturbing bias is seen in the actions of William Russell of Oak Ridge National Lab (ORNL) who completed a major mouse cancer and lifespan study of offspring whose fathers had been exposed to a single high dose (600 r) of X-rays. The findings yielded no treatment effects on longevity, any cancers, including leukemias and other effects. The failure of Russell to publish these data was only revealed some 34 years later when he published it to support a litigation in the United Kingdom (UK) for the defense in a low dose radiation cancer case [63], (see Calabrese and Selby [64] for a detailed evaluation). The reason was given in writing by Russell who indicated that he felt the public could not properly grasp the data and may come to think that ionizing radiation was not as dangerous as Russell believed. Russell was serving as an advisor to the Federal Radiation Council (FRC) at the time of the cancer study completion. Russell decided not to inform the FRC, hiding the data from this federal organization that was the principal advisory body for the President and Congress. Such actions raise important ethical questions concerning Russell's failure to inform the scientific community, FRC as well as the BEAR Genetics Panel (on which he was serving) that was also advising the country on the health effects of ionizing radiation.

2.6. Biological effects of ionizing radiation (BEIR) I: makes crucial mistake that leads to support of LNT

The shape of the dose-response was revisited by the BEIR I [65] Committee. The Committee based their findings on the massive mouse mutation research of Russell. Russell had shown that oocytes display a threshold for mutation at 27,000 fold greater than background radiation. The threshold occurred due to repair of genetic damage that Russell et al. [66] had discovered. However, even though repair was also quite evident in the male, a threshold had yet to be observed in this case. Based on these observations the BEIR I [65] Committee retained the LNT recommendation. This decision of BEAR I has been challenged recently because a major error was subsequently identified two decades later in 1995, showing that the Russell's failed to include cluster mutations in their analyses [67–70]. When the error was corrected according to the Russell recommendations and then reapplied retrospectively to the 1972 BEIR I assessment the findings revealed a threshold for males and an hormetic response for the females [71,72]. Since the LNT model was no longer supported with the corrections, it is now evident that the US Environmental Protection Agency (EPA) based their LNT recommendation in 1975 [73] on a critical scientific error (see section 17 for a more detailed account).

The timing of the BEIR I [65] Report was prescient. When EPA was established in 1970 their strong initial focus was on trying to prevent environmental induced cancer (Elizabeth Anderson, 2022–March 1, 2022 interview with Edward J. Calabrese). Anderson noted that this early EPA leadership strongly believed that the vast majority of human cancers were due to industrially derived environmental chemicals (i.e., pollutants) in the environment. She stated that chemical carcinogens, rather than radiation, was the focus of EPA at that time. In fact, she stated that any concerns with radiation were so limited such as it might be considered the equivalent of "background noise", that is, it wasn't high on the priority scale. The initial goal of EPA was extreme, that is, to eliminate chemical carcinogens and try to create something like the Delaney Clause of the FDA for the newly minted EPA. However, it became pretty clear that if this extreme proposal went forward it would shut down the economy. Thus, the leaders of EPA had to abandon their goal of zero exposure to chemical carcinogens with an "acceptable" risk concept that would be driven by an LNT model since a threshold approach for carcinogens was not an acceptable option. It was off the table. It was at that time that EPA's Roy Albert came to learn of, and appreciate, the option offered by the BEIR I Report [65]. It gave EPA the LNT option for ionizing radiation. However, Albert deftly saw that it could be generalized to chemicals under the assumption that chemical carcinogenesis also was mediated by a mutational mechanism like ionizing radiation. LNT was therefore born within EPA and had its application to both chemicals and radiation. Thus, the timing of the BEIR I Report and the needs of a young EPA to have a risk based way to manage and limit human exposure to carcinogens was created. This story related by Anderson in the interview is consistent with the historical summary provided by Albert [74].

2.7. LNT- the chemical domain

Radiation geneticists led by Muller inspired the idea that chemical carcinogens could also induce irreversible genetic damage initiating the carcinogenesis process. This notion became institutionalized within the scientific community and was adopted by the International Union Against Cancer (IUAC) in August of 1956 based on a 1954 recommendation by the IUAC that a distinction existed between reversible toxic responses and the assumed irreversible actions of mutagenic carcinogens [75,76]. By 1958, the US Food and Drug Administration (FDA) adopted the Delaney Amendment that banned the addition of carcinogens to food, which was partly based on these 1954 and 1956 IUAC policy recommendations [25].

These initial developments on carcinogen regulation relied upon limited scientific foundations and were led by the radiation geneticist community, principally by Muller and his colleague Curt Stern [36–38] and on the chemical side by Wilhelm C. Hueper, a close advisor to Rachel Carson and representative Delaney (i.e., Delaney amendment). The newly formed (1970) US EPA (published in 1975 Federal Register, 41, 29,409 [73]) proclaimed that its belief in LNT was based on the BEIR I [65] (see US EPA [77]) report for ionizing radiation, a report that was authorized by the US FRC in 1970.

Chemical carcinogens were subsequently lumped together with ionizing radiation because both were assumed to act via mutational

[12] Bentley Glass was one of only six senior editors of *Science* at this time and also was a member of the BEAR I Genetics Panel.

mechanisms [74]. Multiple influential publications by Bruce Ames reinforced these viewpoints by asserting "Carcinogens are mutagens" [78]. Linearity was frequently observed in the Ames assay, adding to the LNT perspective, even if the tester strains showing such linearity lacked DNA repair. Likewise, Mantel and Bryan [79] provided an important bio-statistical initiative in modeling cancer risk assessment, recommending an acceptable risk of one cancer per one hundred million people per lifetime. While the actions of Mantel and Bryan [79] were in response to the US Thanksgiving scare by a cranberry carcinogen in 1959, their perspective was not lost on the US regulatory agencies once the vast environmental regulatory initiative gained political, economic, and scientific strength in the mid-1970s [26,34,80]. Biostatistical modeling was refined subsequently to incorporate the concept of additive to background that insured linearity. However, it was later revealed to be based on an incorrect assumption that the mechanism of mutagenesis was identical between control and treatment groups for the same tumor type in the same organ [81,82].

Within two decades, a transformation in cancer risk assessment had therefore occurred that involved moving from a threshold to a linear dose-response model. These evolving activities interfaced with a body of emerging yet substantial epidemiological literature that sought to validate both the linear genetic damage predictions of the NAS and ongoing linear-based estimations of cancer risk. In general, data from the epidemiology studies revealed important methodological limits in detecting adverse effects that were reminiscent of a threshold effect and depended on endpoint type, background variability, and other factors [83]. Nonetheless, both the belief in LNT and the conservative approach toward risk assessment at the time (e.g., the Precautionary Principle) swept the toxicological, epidemiological, biostatistical, legal and regulatory communities from the mid-1970s onward to become the dominant signature of the environmental revolution, which largely started with the BEAR I Genetics Panel of 1956 [54] and were solidified by publication of Rachel Carson's *Silent Spring* of 1962.

In a theoretical sense, the LNT was founded in the 1950s on the belief that a mutagen (either ionizing radiation or mutagenic chemical) was required to mediate a carcinogenic response from initiation to tumor development and that the size of the carcinogenic response was in direct proportion to the dose of the mutagenic carcinogen—the relationship between mutagenic dose and carcinogenic response was linear. The epidemiological perspective, even if it could not confirm LNT predictions at low doses, became linked to the Precautionary Principle that sought to ensure protection of the most vulnerable subgroups within the population. Epidemiology would defensively claim that each individual might display a carcinogenic threshold but the population would not. The "carcinogens-are-mutagens" concept of Ames however lost some of its initial persuasiveness as more testing revealed that mutagenic effects provided only partial support for the LNT, not infrequently showing threshold or hormetic dose responses depending on the biological model, endpoints measured, total dose administered, dose rate and other factors. Furthermore, much evidence emerged that the responses to some carcinogens were mediated via non-mutagenic mechanisms, suggesting non-stochastic threshold responses. These complex toxicological and epidemiological frameworks had little influence on environmental regulations, which was driven by an overriding precautionary belief that lower exposure was always better. Although the expressed regulatory goal of EPA would become zero carcinogen exposure, practical considerations emerged with the adoption of the concept of a *de minimis* risk of one cancer in a million people over a lifetime of 70–80 years.

This brief summary of the historical foundations of LNT is extremely troublesome as many key foundations that have been used to support LNT have been shown to be based on scientific errors, profound biases, ethical failings and scientific misconduct at the highest level of scientist and scientific organization (Fig. 1). These findings indicate that the historical foundations of LNT are highly corrupted and do not support LNT principles and practices.

3. Relationship between biological function and the number of atoms needed to induce it

Hutchinson [84,85] assessed the number of atoms required to affect enzymatic and other functions of liver cells. A relatively large number of atoms are required to affect a response even when high biochemical specificity is necessary for a reaction to occur. Even at its most sensitive level of activity, the liver cell, and probably most other cell types, requires more than 10^4 atoms/molecules per cell to significantly affect a response. In the case of non-nutritive toxic agents, thresholds are a

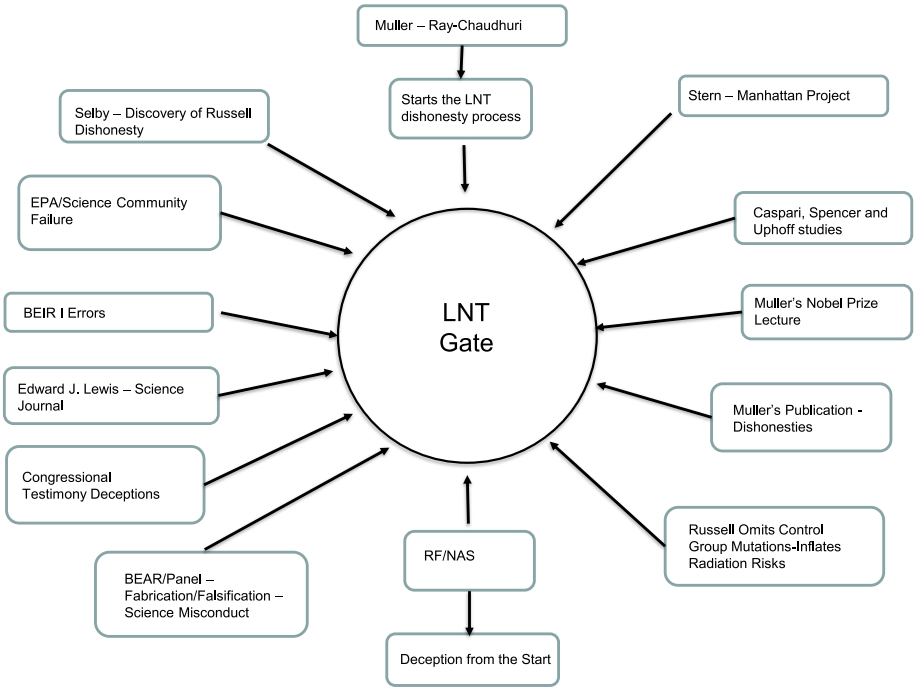

Fig. 1. LNT: Summary of historical errors, biases, and misrepresentation of the research record.

dominant feature of biological systems. Dinman [86] concluded: "To believe that such molecules cause an undesirable effect disregards the presence of a multiplicity of interfering substances. Such thinking also does not take into account the fact that the dose of a foreign atom may be related to the probability of its interacting with an available active site, or that similar probability governs the answers to the question of whether interactions will occur at discrete topographical loci upon a structural or functional molecule (or on a possible precursor). While the construction of stochastically sound models is remote, the reasonableness of the hierarchy of cellular element concentrations as these relate to metabolic function suggests that a threshold for biological activity exists within a cell at 10^4 atoms."

The arguments of Hutchison [85] and Dinman [86] were supported in subsequent assessments by Claus [87] and Jukes [88]. In a similar fashion and based on the Dinman analysis, Friedman [89] estimated that the minimal number of inducing molecules for various potent carcinogens were 8.6×10^{15} molecules/kg body weight. In 1980, Preussmann [90] applied this concept of thresholds for chemical carcinogens to the data of Mohr and Hilfrich [91]. Kidney tumors were induced in rats via a subcutaneous injection of diethylnitrosamine (DENA) using eight different single dose treatments ranging from 1.25 to 160 mg/kg. He reported 11/20 kidney tumors in the single dose of 100 mg/kg and 1/20 kidney tumors at 1.25 mg/kg (i.e., 0.3 mg/rat). The control group showed no kidney tumors. The 1.25 mg/kg exposure, which was assumed to be carcinogenic, corresponded to about 2×10^{18} molecules/rat or approximately 10^{16} molecules/kidney. Based on a standard weight of the kidney, there were about 10^6–10^7 molecules of DENA/-kidney cell at this dose. The range of 10^6–10^7 molecules/cell was about 100 to 1000-fold greater than the postulated theoretical threshold of 10^4 molecules/cell of Dinman. Similar estimates of benzo(a)pyrene molecules have been reported for mouse epidermal tumors [92]. These data indicate that it is highly unlikely for one ionized/unionized molecule, atom or subatomic particle per cell to produce a mutation, let alone a lethal event.

4. The Second Law of Thermodynamics makes LNT highly improbable

The notion that one (or even more than one) mutagenic particle will produce genetic damage is not only unlikely but also unreasonable given the vast numbers of mutations that are now known to be produced and repaired successfully in a single cell each day [16]. It also stands to reason that the capacity of a cell to repair its DNA is not infinite and thus some higher exposure level would surely exceed its repair capacity, resulting in the accumulation of potentially pre-cancerous genetic lesions. Such reasoning gives rise to the concept of a "threshold" response existing for mutagenic damage and contradicts both the applicability and utility of the LNT model at exposures that are sub-threshold. Thus, the capacity of a cell to repair DNA damage before the damage can affect permanent carcinogenic alterations produces a "threshold" response that depends on both the cellular capacity to repair DNA and the rate at which the repair occurs.

While a threshold response may be the result of the cellular repair response to DNA damaged by mutagens, as just discussed, a threshold effect also may be a result of the thermodynamics (Second Law) that largely determine the reactivity of mutagens before they react with DNA and other non-critical target molecules in the cell. The Second Law of thermodynamics states that as energy is transformed it is progressively wasted (i.e., entropy is increased). Thus, it provides a foundation for assessing whether such a threshold is theoretically possible. It is known that each interaction between molecules does not result in a chemical reaction (e.g., biological effect-mutations). Mutagenic/carcinogenic compounds need to provide the necessary activation energy to react with the critical receptor molecules, yielding DNA-adducted chemical products. Since chemical bond energy is the "exclusive source of utilizable energy in biological systems, there is a minimum activation energy and a minimum net free energy above zero for all cellular reactions including the induction of cancer" [93].

The covalent binding of mutagens to DNA affects DNA alterations (i.e., mutations) and can initiate the process of carcinogenesis if they are unrepaired. A thermodynamic analysis of this process requires a specific energy threshold to be exceeded to affect the induction of a genetic change/aberration. Koch [94] estimated that the activation energy might vary between 16 and 42 kj/mol for biochemical reactions *in vivo*. The minimum net energy required to induce a single-strand break was estimated to be at least 17 kj/mol [95], supporting the existence of a threshold. Chemical reactions therefore occur only where a sufficient number of molecules display kinetic energy, collide with each other, yield an intermediate product, and then transform to final products. The formation of a reactive intermediate represents a random event within an environment of vast numbers of molecules. The actions of a carcinogen represent a stochastic process involving massive numbers of molecules and targets, many of which are non-critical. Based upon the above thermodynamic factors, Koch [94] calculated exposure-effect thresholds from consumption of drinking water (2.5 L/day), assuming 10^2-10^4 molecules/cell. The Maxwell-Boltzmann equation estimated that one molecule per 10^4 will become activated with an activation energy of 40 kJ. At such a rate of activation efficiency, Koch [94] concluded that the induction of biological effects (e.g., mutation) would not occur below such thermodynamic driven thresholds. He then applied this framework to ten environmental pollutants, including benzene, chloroform, and DDT, yielding an estimated biological effects threshold for drinking water. This approach provides a thermodynamic-based approach to estimate biological effects, including the process of carcinogenesis. It is based on physico-chemical principles and can be used to test the reliability of statistical modeling.

Since chemical reactions are subject to entropy and free energy constraints, Schaeffer et al. [96] indicated that mutational effects display activation and free energy requirements as well as entropic dependence. Since entropy represents a statistical estimate of system disorder, these reactions display the mass requirements of chemical reactions. Thermodynamic principles therefore indicate that the LNT is both chemically and biologically highly improbable at low doses. Koch [94] concluded that such outcomes provide not only a theoretical but also a practical means of translating toxicological findings into valid risk assessments. This perspective illustrates that it is extremely unrealistic to think that even at rates of thousands of mutagenic molecules per cell effects on DNA will cause mutations, thereby failing to support LNT as a likely dose-response model.

The US EPA drinking water standard for benzene is 5 μg/L and has an estimated 1×10^{-5} lifetime risk of developing leukemia based on LNT models. At this standard, 1 L of water contains an estimated 8×10^{15} benzene molecules. The number of molecules that one could be exposed to at this standard is further increased by consuming 2 L/day over an 80-year lifespan. For the vast majority of regulated carcinogenic and non-carcinogenic agents in drinking water, the estimate of acceptable exposures for an 80-year lifespan is usually in the range of 10^{14}–10^{20} molecules/day.

The two previous sections (#3 and #4) demonstrate that LNT is extraordinarily unlikely based on both empirical evidence as well as more theoretical estimates from the Second Law of Thermodynamics. These two approaches are complementary and provide a foundation to assess DNA adducts and low-dose linearity.

5. Pharmacokinetic and pharmacodynamic factors and carcinogen dose response

Mutagen genetic damage is strongly dependent on and affected by the pharmacokinetic processes of absorption, distribution, metabolism, storage and excretion. These processes involve the interaction of component parts of the biological system with unique physico-chemical properties of the mutagenic molecules, which may include both the

parent mutagen and/or possible mutagenic metabolites. The occurrence of a mutagenic event having relevance to a carcinogenic outcome will be affected by the following factors: (1) the number of mutagenic molecules that survive the pharmacokinetics and can reach the DNA of targeted cells, (2) the thermodynamic parameters that affect the activation and covalent binding of the mutagenic molecules to DNA, and (3) the biological capacity and rate of DNA repair in the target cells. While each of these factors alone tends to reduce the likelihood for any single mutagen molecule (the smallest dose possible) producing a mutational event, the combination of all three factors would only further greatly reduce the likelihood, virtually eliminating the possibility. To increase the probability of a mutational outcome, it would require continually increasing the number of mutagens (i.e., dose or level of exposure) until some threshold value is exceeded and the likelihood of evading these barrier-like factors is sufficient to register a mutational event. Essentially, this describes and lends rational support to a threshold dose-response model where some low-dose value must first be exceeded before a mutagenic effect can be observed.

5.1. DNA adducts – a method to estimate thresholds and/or linearity?

A mechanism-based method for estimating the threshold of a carcinogen involves DNA adducts as biomarkers for chemically induced tumors. Williams et al. [97–99] quantified the biological processing of several chemical carcinogens starting from the initial exposure, through the spectrum of pharmacokinetic/pharmacodynamic processing, the induction of DNA adduct formation, the location of adducts, the efficacy of DNA repair, and finally the rate of neoplastic transformation, including the number of mutations, the development of tumor microvasculature, and tumor suppressive elements. This information led to the derivation of a cell-based mechanism of action for what was designated as a safe-exposure level (SEL) for DNA-reactive chemical carcinogens. This approach was based on the binding of a chemical agent to DNA, producing less than one adduct per 10^9 nucleotides.

Starting with an oral exposure, stable pro-carcinogenic agents are subject to degradation via chemical and biological processes, including gut microflora. After gastrointestinal absorption, the remaining pro-carcinogens are transported by blood to the liver where they undergo a first-pass extraction and metabolism.

The lesser portion of the pro-carcinogenic dose that is not taken up by the liver enters the systemic circulation, usually in a highly diluted manner. These so-called "escaped" molecules distribute to many organs and their cells, further diluting the dose [98]. The only cells at risk for initiation (i.e. cancer development) are proliferating stem cells [99] and these cells typically comprise <1% of the cell population in any tissue. In addition, these cells contain an active chemical extrusion process involving p-glycoprotein that pumps hydrophobic agents, such as DNA-reactive electrophiles, out of the cell and/or prevents their entry, thereby causing a further reduction in the exposure of carcinogens to critical cellular targets.

The greater portion of the pro-carcinogenic dose that is taken up by the liver is either metabolized into non-reactive species (~80%) or bio-activated into electrophilic carcinogens (~20%) that can react indiscriminately with cytoplasmic and nuclear nucleophiles, including RNA, amino acids, peptides, proteins, lipids, as well as DNA [97–99]. However, because DNA is the only nucleophile with carcinogenic potential, the reactions of other nucleophiles (i.e., non-DNA nucleophiles) with electrophilic carcinogens will essentially quench or neutralize the carcinogenic potential of those carcinogens, thereby reducing the likelihood of a carcinogenic event even further. It should be noted that the covalent binding of these bio-activated species is 100 fold greater in the cytoplasm than nucleus. Furthermore, most DNA in most cells is shielded most of the time from such reactions by a protective coat of nucleophilic proteins (histones), making the likelihood of an electrophilic reaction with DNA even more remote. Considering the fact that all gene-encoded DNA (about 9×10^7 base pairs) in the genome represents only a small fraction of the total genome (about 3.2×10^9 base pairs) means that roughly only 2% of genomic DNA is composed of genes and therefore susceptible to adduct formation and mutation [98,99]. Conversely stated, 98% of the already exceedingly small number of surviving electrophiles that actually form adducts with DNA will not mutate a gene. Finally, the likelihood of a mutation is still further diminished when one considers that multiple genes, upwards of about six to eight, need to be mutated (perhaps in a defined sequence) to result in the development of a tumor [100].

DNA adducts are clearly produced at low levels of exposure as has been reported by Williams et al. [100]: Aflatoxin - 70 adducts/10^8 nucleotides; DMN – 360 and 5 adducts/10^9 nucleotides for N^7 and O^6 methylguanine adducts, respectively; and 1 adduct/10^8 nucleotides for meIQX. However, there exists no knowledge of the level at which adduct formation translates into a biologically or toxicologically significant event. According to binding studies, spontaneous DNA damage occurs at a rate of about 1 lesion/10^6 nucleotides, indicating that the rate of spontaneous DNA damage is about 10–100 fold greater than what has been reported above by Williams et al. [100] for low dose exposures of several carcinogens.

To develop the above analysis a bit further, it would seem reasonable to conclude that the formation rate of 1 adduct/10^{11} nucleotides provides a threshold below which no biologically significant event may be observed [100]. Consider that at the rate of 1 adduct/10^9 nucleotides, only about 3 adducts/cell are formed. With only about 2% of the genome being composed of genes and functionally active (as previously mentioned), the likelihood is strong that none of the three adducts will even occur in the DNA regions coding for DNA products. Thus, of the very small proportion of gene adducts that may actually occur, an even smaller proportion will be mutagenic because the overwhelming majority of these gene adducts will be repaired. Taking the analysis even further, not all mutations are of equal biological relevance. Only mutations in certain locations of susceptible genes can alter gene function. The result then is that many mutations can be without critical public health and/or medical significance. For example, the vast majority of mutations of the p53 tumor suppressor gene did not significantly affect the amino acid structure of the protein [101]. Another factor affecting the development of a tumor is the need to circumvent host resistance and to form a neovasculature that enables the tumor to transform into a neoplasm. According to Williams et al. [100], the probability of progressing from a pre-neoplastic to a neoplastic tumor is $\leq 1/1000$. This capacity to progress is largely a function of dose, which can affect both the replication potential of initiated cells and their tumor-promoting stimuli, many of which are inflammatory in nature. These tumor-promoting/inflammatory factors are expected to be far less effective at low than high doses.

Based on the above mechanistic approach, Williams et al. [100] isolated specific stages of the process of carcinogenesis, including cell proliferation and the induction of numerous preneoplastic and progressive hepatocellular lesions. For each of these steps, they report a threshold response. These findings led Williams et al. [100] to conduct a cancer risk-assessment study using the frequency of DNA adduct formation as a molecular marker and predictor of cancer risk. Results from the study indicated that the LNT model was inadequate and should be changed to a mechanistically based threshold model. Taking a slightly different approach, Thomas et al. [102] systematically identified various biological functions that interfere with (eliminate, delay or reduce) the tumor-forming potential of carcinogens and, in so doing, argue in support of a threshold model. Examples of these tumor-interfering functions included the scavenging of electrophiles, cellular effluence, DNA repair, apoptosis, autophagy, silencing via DNA-damage-triggered replicative senescence, and the immunological elimination of precancerous cells.

The above argument is the result of several decades of detailed toxicological investigations [103]. It challenges the long-held adherence to linearity that, as stated by Fahmy and Fahmy [104], "there seems to be no such thing (i.e., thresholds) as far as mutations are concerned."

Bruce Ames [105] further supported the LNT perspective with the statement: "It is worth emphasizing that one molecule of a mutagen is enough to cause a mutation and that if a large population is exposed to a 'weak' mutagen it may still be a hazard to the human germ line, since no repair system is completely effective, there may be no such thing as a completely safe dose of a mutagen".[13] These precautionary statements have lost scientific standing over time. From the stochastic, pharmacokinetic, pharmacodynamic and thermodynamic perspectives, as evidenced above, this type of linear conceptualization is fundamentally flawed.

A recent assessment by Kobets and Williams [99] of animal long-term dose-response experiments in chemical carcinogenicity was conducted using a broad range of dosages (i.e., >3 dosages, at least 10-fold dosage range, and the highest dose being well tolerated), continuous repeat dosing for a substantial portion of the animal's lifespan (e.g., at least 1 year for rodents) and with observations for ≥2 years. The results from studies of 14 DNA-reactive and 7 epigenetic carcinogens demonstrated a threshold effect for tumor development. According to Kobets and Williams [99] "… the totality of the abundant biological and mechanistic evidence unequivocally supports the existence of No Observed Effect Levels (NOELs) [i.e., thresholds] in experimental carcinogenesis, for both for tumors and antecedent effects for both DNA-reactive and epigenetic carcinogens." This definitive perspective supporting a threshold response was then coupled by these authors to a conclusion that there are no high quality empirical experimental findings with mechanistic explanations which reveal the LNT model to be a valid dose-effect model for carcinogenic chemicals.

5.2. *Dose-dependent transitions and underlying mechanisms*

The issue of dose-dependent transitions in toxicity and how such changes could be mechanistically explained has significant risk assessment implications. Processes that affect such dose-dependent transitions include: gastrointestinal tract or respiratory absorption; tissue distribution as affected by protein-binding and active transport systems; chemical transformation, including bio-activation and detoxification; receptor interactions; and/or tissue- DNA-repair processes; as well as altered homeostasis.

One example of a dose-dependent transition is the induction of nasal tumors in rats following their respiratory exposures to propylene oxide [106,107]. In this example, both the development of tumors and the proliferation of cells in rat nasal respiratory epithelium displayed threshold effects and thus contrasted sharply with the linear relationship observed for hemoglobin- and DNA-adduct formation in the same tissue. The observation that the actual development of tumors could be explained more directly by the threshold response of cell growth than the linear response of adduct formation (Fig. 2) not only indicates that the LNT model could not account for the development of nasal tumors but also questions the real importance and relationship of DNA adduct formation to tumor development, especially at low levels of exposure.

In addition to propylene oxide, formaldehyde is another carcinogen that was studied to better understand the effects of dose on cell proliferation and, in this case, the induction of nasal squamous cell carcinoma in rats. It was found that doses associated with cancerous responses were also toxic and produced cell death, which was closely followed by the stimulation of cellular re-growth referred to as regenerative cellular proliferation (RCP). In a cancer bioassay, Monticello et al. [108] exposed rats to five concentrations of formaldehyde (0.7, 2.0, 6.0, 10.0, and 15.0 ppm) and, at various times afterward (days 1, 4, and 10, and weeks 6, 13, 26, 52, and 78), determined RCP at multiple locations in the

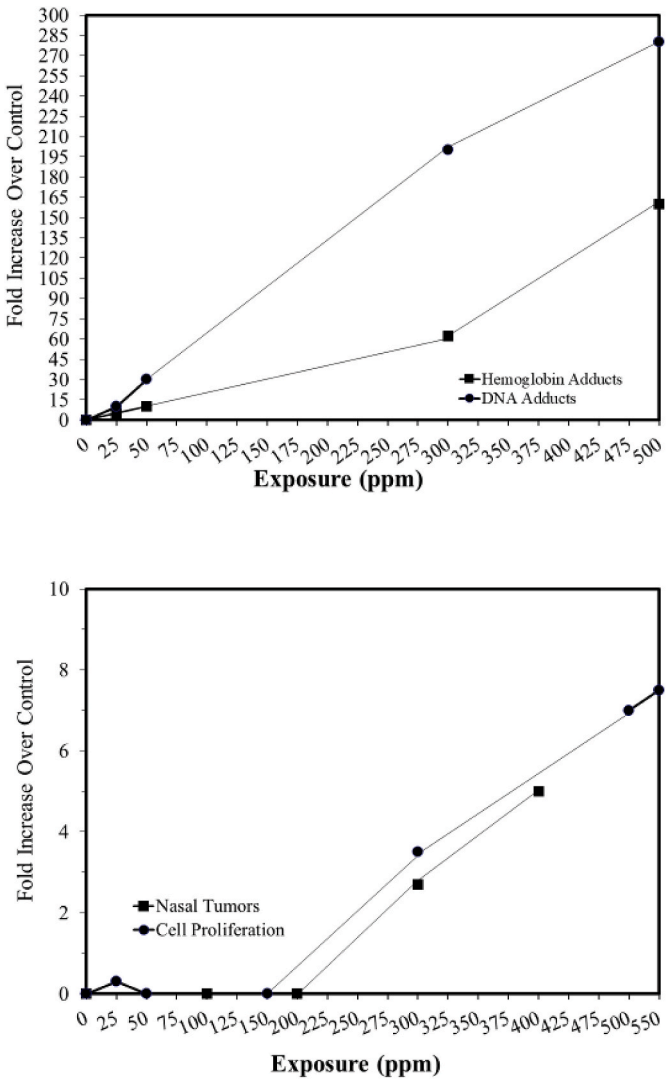

Fig. 2. Dose-dependent transitions for nasal respiratory epithelium from rats exposed by inhalation to propylene oxide (Source: Based on Slikker et al. [106,107].

nasal tissue. Formaldehyde was found to induce a J-shaped dose response in RCP that was actually below the control response for the two lowest concentrations (0.7 and 2.0 ppm). At these low formaldehyde concentrations, the incidence of nasal tumors was observed to be less than that of the control group, correlating with the below-control values for RCP. Such findings support the hypothesis that the incident risk for tumor growth is associated with the cell replication rate, as was true for the propylene oxide study.

The decrease in the cell proliferation rate at low concentrations of formaldehyde is consistent with hormesis, which is strongly indicated by the J-shaped biphasic RCP response induced by low formaldehyde concentrations [109]. It is well established by definition that low hormetic doses are non-toxic (i.e., they occur in the non-toxic dosing range below the toxic threshold) and enhance cellular metabolism in ways typically beneficial to cell survival, such as by increasing DNA repair, increasing autophagy, and increasing ATP production. Given that an hormetic dose is non-toxic and may enhance DNA repair and cellular bioenergetics, it is reasonable to surmise that cells in the low dose hormetic range of formaldehyde will have fewer DNA cross links than the control cells (as is evinced by the J-shaped dose response) and will be healthier than controls, with enhanced survival value.

[13] Ames would later focus his attention on the occurrence of plant based dietary mutagens rather than those of an industrial origin. This is because human exposure to mutagenic agents is dominated by dietary exposures comprising approximately 99% of typical human mutagenic exposures.

6. Additive to background hypothesis

Cancer risk assessment is highly dependent on the assumption that cancers induced by chemicals and ionizing radiation occur via the same mechanism as background cancer. This was a key assumption made by Crump et al. [82] for quantitative risk assessment, which led to the integration of the additive-to-background concept into the spectrum of competing cancer risk assessment models. The EPA later adopted the additive-to-background assumption, which replaced an independent-of-background assumption by the early-1980s and remains in place today [81]. The evidence supporting the adoption of additive to background was very limited, with little reference support. Nonetheless, the concept proved compelling, most likely because of its seemingly natural fit with LNT. An early assertion by Crump and colleagues in 1976 alluded to this point. That is, "if the carcinogenesis by an external agent acts additively with an already ongoing process then under almost any model the response will be linear at low doses [82]." A decade later, the EPA [110] said virtually the same thing: "if a carcinogenic agent acts by accelerating the same carcinogen process that leads to the background occurrence of concern, the added effect of the carcinogen at low dose is expected to be virtually linear". The key assumption here is that both spontaneous and induced cancers need to be biologically indistinguishable for the concept of additive to background to be valid. That is, the mechanisms for spontaneous and induced cancers must be precisely the same—and not just similar molecular variants of each other. The fact that pathological studies could not definitively prove either the sameness or difference between spontaneous and induced cancers [111] means that no definitive evidence exists in support of additive to background and that it remains an unproven concept. Since linearity at low doses also remains unproven, it would seem not only disingenuous but also illogical to apply one unproven concept (i.e., linearity at low doses) in support of another (i.e., additive to background) or vice versa.

The problem with the additive to background assumption was that little was known about mechanisms of carcinogenesis in the mid-1970s. It was principally conceptual, with the major belief being that "carcinogens were mutagens". However, in the mid-1980s the oncogene revolution was born, linking mutations to pathways and tumor formation. These research developments were not only very important for environmental carcinogenesis but also dominated the search for chemopreventive/antitumor agents that could be used in cancer chemotherapy. Despite these important developments in the oncogene area, the additive-to-background assumption that was put forth in 1976 was not formally tested until 2018 when Calabrese [81] published a detailed evaluation that employed the methods and findings from molecular toxicology, including oncogene activation/mutation, gene regulation, and molecular pathway analysis. Essentially, results from studies that encompassed 45 carcinogens, over 13 mammalian models, and a wide range of tumor types indicated that different mechanisms were involved in mediating carcinogen-induced and spontaneous tumors. In that evaluation, carcinogen-induced tumorgenicity showed a vastly different spectrum of tumor oncogenes than observed in the same tumor type of the control groups. This demonstrates that a key assumption used by EPA for quantitative estimates of cancer risks at low dose is not supported. This new assessment and interpretation extend and are supported by a report on "The Scientific and Practical Basis for Thresholds in Biology" that genotoxic carcinogens that induce tumors via different mechanisms (i.e., no assumption of additive to background) than occur in controls can be considered to act via a threshold process. Such a conclusion has profound implications for the risk assessment process [112] - see Ian Purchase discussion). Furthermore, exposure to carcinogens can markedly alter the occurrence of spontaneous tumors, reducing/preventing some background tumors and further challenging the concept and meaning of background tumor incidence [81]. Although this analysis raised many new questions for cancer risk assessment, it established that the additive-to-background assumption is not a general one, is often incorrect, should not have been adopted by EPA for cancer risk assessment, and lacks scientific validity.

7. Promotion is not a tumor stochastic process

7.1. Promotion is a threshold process

Carcinogenesis is a multi-stage process involving initiation, promotion and progression. In cancer bioassays the induction of initiating events, such as mutations, typically occur at lower doses than the induction of inflammation and other related tumor-promoting actions such as hyperplasia [113,114]. In addition, promotion can be essential for tumor development, often requiring prolonged, high-dosed treatments with tumor-promoting agents. Removal of the promoting stimulus frequently leads to the regression of a high proportion of initiated tumors. Significant heterogeneity characterizes the development of tumors that result from different levels/degrees of initiation in the targeted cells. For example, promoter-dependent papillomas progress from skin epidermal cells with "low levels" of mutations (i.e., low doses and few mutations). In contrast, promoter-independent tumors can develop from cells with "high levels" of such mutations. The dosing levels have also been shown to affect whether an initiated cell progresses in a monoclonal fashion or can be further promoted and transformed into a multi-clonal framework of tumors. These developments indicate that an initiating dose in carcinogenesis may not be sufficient to activate other essential processes needed to complete tumor development. These findings support a threshold rather than LNT framework and are consistent with a new somatic mitogenic/clonal expansion (MSM) theory on cancer as detailed by Bogen [115]. Accordingly, cancer is a multistage process that is mediated by inflammation and involves somatic mutations and clonal expansion. Since the non-inflammatory doses of carcinogens are typically low-dose exposures, the MSM theory would predict low and/or negligible risk in such exposure conditions.

7.2. Carcinogen-induced immune suppression

Another aspect of the tumor promotion/progression process is that many carcinogens at high doses induce immune suppression in the chronic bioassay that may contribute to enhancing tumor yield and decreasing time to tumor, markedly affecting cancer risk assessment and even possibly the designation whether an agent is classified as a carcinogen. Powerful immune suppression affecting T-cell mediated immune responses against tumors have been reported for well known carcinogens including mycotoxins, such as aflatoxin [116] and fumonisin [117], arsenic [118–120], multiple polycyclic aromatic hydrocarbons such as benzo(a)pyrene [121,122] and dimethylbenzo(a)pyrene (DMBA) [123], ethyl carbamate [124], ultraviolet A radiation [125], and asbestos [126]. The effect of these carcinogens on these immune functions is also a threshold process. No attempt has been made in the regulatory agency carcinogen risk assessment process to experimentally clarify and "decouple" the capacity of the carcinogen to induce mutation and suppress immune function. The high dose-few doses chronic bioassay improperly integrates these two processes within a risk assessment-low dose extrapolation framework. It has been widely shown that the high dose immune suppression response can be a strong driver for tumor promotion and progression. This could lead to an incorrect LNT interpretation, with the high dose cancer response having a dominating role in the low dose statistical model risk estimates. These findings have the potential to challenge the past five decades of cancer bioassay-based risk assessments for numerous immune suppressive agents.

7.3. Use of highly inflammatory rearing practices in the cancer bioassay

Another built-in promotional bias in the chronic cancer bioassay is ad libitum feeding and the progressive development of obesity along with lack of exercise. These are well known factors that lead to the

occurrence of age-related higher inflammatory biomarkers that enhance the progression of tumors. Coupled with the massive doses that are employed, the chronic bioassay becomes a weaponized tumor induction instrument that has little to do with providing realistic estimates of potential cancer risks. More realistic changes in the amount of food and the daily temporal restriction of food consumption could radically change the outcome (i.e. reduced risks) seen in chronic bioassays [127]. The chronic bioassay has long been a driver in the application of regulatory agencies for carcinogen risk assessment. It is time to not only change current chronic bioassay practices but to also reassess risks derived from past studies in which grossly unrealistic rearing practices distorted cancer risk estimates. All completed cancer bioassays using regulatory based decisions need to be re-evaluated in light of these new developments that may affect past cancer risk estimates, providing a type of regulatory evaluation audit. Evaluation protocols would be needed for such retroactive regulatory evaluation process audits.

8. Carcinogens: latency and dose response

Tumor incidence is the principal way in which dose is used to evaluate carcinogenic responses. However, Hermann Druckrey published a series of papers [128–131] establishing a relationship between the dose and the time required to detect tumors, that is, the tumor latency period. As a new concept, it has the capacity to develop an estimate of cancer risk in a manner complementary to the standard method involving dose and tumor incidence. The key observation was that an inverse relationship exists between dose and tumor latency, that is, lower doses require longer times for tumors to appear. This concept implies that a sufficiently low dose could have a tumor latency period longer than the normal (i.e., average) lifespan of the experimental species. In a statistical sense, groups treated with such low doses would have tumor incidences indistinguishable from controls. An interesting consequence of this concept is that the linear dose-response model could be accepted as valid and still display a "practical threshold" for carcinogens. This proposal by Druckrey represents a type of toxicology-based compromise between the LNT and threshold dose-response models.

The Druckrey tumor-incidence concept (dose relates inversely to latency) generated considerable worldwide interest. In the former Soviet Union, the concept of dose-latency was repeatedly reported (e.g., Suss et al. [132]; Yanysheva and Antomonov [133]) and finally accepted in risk assessment practices by the late 1970s for the carcinogen benzo(a) pyrene. It was asserted that any carcinogenic effect of benzo(a)pyrene would be observed only after the normal life span of the species had been exceeded by a considerable time. In fact, in a number of the above cited studies low doses actually enhanced immune cell function, suggesting an hormetic dose response.

Such an idea also resonated with some leading researchers in the US, including Hardin Jones at the University of California at Berkeley, an expert in human aging, especially with respect to the effects of ionizing radiation. He and his colleague Alexander Grendon published several papers that integrated and extended the findings of Druckrey and others, deriving a predictive bio-mathematical model for tumor incidence [134]. If the dose of the carcinogen were decreased by a factor of 1000, the latency period would increase by a factor of 10. As a result of the meta-analysis of 11 epidemiology studies on asbestos exposure and lung cancer, Enterline [135] applied the Druckrey concept to the field of occupational cancer.

Even though Druckrey's concept was replicated and extended to include a range of compounds (via animal models and epidemiological meta-analyses) it was nevertheless rejected by US regulatory agencies and thus failed to be integrated into the processes of hazard- and risk-assessment. Why would this be the case? Historically, Occupational Safety and Health Administration (OSHA) conducted massive hearings in 1978 on carcinogen policy [136] where the dose-latency concept of Druckrey and Jones was considered. However, neither Druckrey nor any others who had published findings in support of the dose-latency concept testified. Jones was expected to offer testimony but unexpectedly died less than two months prior to the hearings. His relevant papers were entered into the record and commented upon, especially by those who were opposed to having this concept affect carcinogen risk-assessment policy. Without his presence to personally defend his position, there was little likelihood that the dose-latency position would prevail, especially after several notable opponents (e.g., David Hoel, Umberto Saffiotti, Richard Peto and Marvin Schniderman) exerted efforts to block its acceptance. The most substantive criticism was by Hoel [137], who argued that an inverse relationship between dose and latency was not an unexpected finding as it was readily predicted with the assumptions of stochastic modeling. Although mean latency could far exceed normal lifespan by as much as 10–20 fold, modeling efforts based on the Poisson distribution predicted the occurrence of some tumor incidence within a normal lifespan. As a result, the concept of dose-latency was dropped from both the cancer risk-assessment process and the hazard-assessment testing scheme.

In the intervening years, several lines of research have supported a Druckrey/Jones-like perspective. In discovering that a much higher dose is required to promote tumor growth and decrease tumor latency than is needed to initiate tumor growth, it became apparent that the converse was also true, i.e., a much lower dose may still initiate tumor growth but will also have decreased tumor-promoting potential and increased tumor latency. Although initiation appears to be a stochastic process, promotion is a threshold dose-response phenomenon. Therefore, the idea of dose affecting latency has a solid scientific foundation, does not have to be a stochastic process, and offers a rational biological explanation for the existence of a carcinogen-induced "practical threshold" in carcinogenesis.

Criticisms presented at the 1978 OSHA hearing related mainly to independent stochastic processes that affected the initiation and progression stages of carcinogenesis but not the promotion stage nor its impact on tumor latency. Since then, however, new findings have revealed that inflammation promotes tumor growth and decreases latency (the time to tumor) and also that higher promoting (rather than lower initiating) doses of carcinogens are inflammatory. The logical deduction from these findings is that high-promoting doses are inflammatory and decrease the time to tumor, effectively yielding more tumors sooner to somewhat resemble a linear-like dose-response construct. Conversely, low-initiating doses, which are neither promoting nor inflammatory, increase the time to tumor, yielding fewer tumors later to somewhat resemble a threshold-like dose-response construct [113].

Furthermore, many of the experimental studies used by Jones and Grendon to support the dose-latency concept involved single-exposure protocols. In these cases, the single dose had to act as a complete carcinogen, affecting initiation, promotion and progression. Since a single dose had to be high enough to affect all these different processes, the relevance of these high-dose, single-exposure protocols to low-dose community risk assessments seems problematic and possibly untenable. Nevertheless, it is still possible that a single exposure, even at a non-promoting dose, could cause cancer during a normal life span. In this case, the dose would have to be administered at a time when endogenous conditions are conducive to promotion. For example, this may happen when female Sprague Dawley rats are treated with carcinogens at a time when mammary tissue is massively proliferating during days 48–52 [138], resulting in the profound shortening of the tumor latency period and even appearing linear-like in response to dose.

The relationship/interaction between carcinogen dose and tumor latency is complex and dynamic in that the biological disposition of any carcinogen by any organism will be influenced not just by the dose but also by the chemical and physical properties of the carcinogen as well as the various endogenous and exogenous factors affecting the organism. Nevertheless, given any carcinogen and any organism under any set of conditions, the general tenet is that high doses shorten latency and appear more linear-like while low doses lengthen latency and appear more threshold-like. Since exposures to environmental carcinogens are

mostly low-dose and thus associated with long tumor latencies, the dose-latency concept would argue for a threshold rather than linear dose-response model to be incorporated into the cancer risk assessments for environmental carcinogens.

9. Hormesis challenges LNT

9.1. Historical foundations

In the introduction to the 1975 book *Heavy Metal Toxicity, Safety and Hormology*, Donald Luckey [139] and his colleagues argued that hormesis presented a serious challenge to the LNT dose-response model and would have implications for cancer risk assessment.

> Quote from Luckey [139]: "Agents which are found to cause stimulation when given in small quantities are called [called] *hormetics* and the action is *hormesis*, taken from Southam et al. [and Erhlich] (1943). Understanding [Understandings] the extent of this phenomenon is essential before worldwide committees and legislative bodies make recommendations which consider only toxic actions …"

The dose-response debate during the early 1980's was not hormesis versus LNT *per se*, but threshold versus LNT. The regulated industry for chemicals and radiation supported the less conservative threshold dose-response model whereas the EPA had established the LNT model for its estimation of cancer risks from carcinogens. Since using the LNT model for risk assessment and risk management decisions proved to be very costly, the regulated industry sought to convince the EPA that the threshold model would be the better model for the purpose of cancer risk assessment. A practical problem for industry was that the EPA evaluated each chemical in the chronic bioassay (i.e., the two year rodent cancer bioassay) on its own merits, assessing whether the data best fit a linear or threshold model. Although this might have appeared to be an objective approach, the problem was that too few doses had been used to differentiate which model best fit the data. Compounding this problem was the fact that these few doses were also administered at very high dose rates, that is, at the maximum tolerated dose (i.e., the highest dosage that would not cause frank toxicity and not diminish body weight by more than ten percent) and one half of that value. Because the chronic rodent bioassay produced very few data points, it was possible that either a linear or threshold model could fit the data set. The EPA would invariably default to the most conservative dose-response model. For the regulated community, however, there appeared to be no practical way around this risk-assessment dilemma. The procedural rules of the risk assessment process were biased toward using the LNT model. Thus, the "scientific" deck was stacked to yield a predetermined result.

As a result of this situation, some attention was directed toward the hormetic dose-response model. Firstly, it was considered to be easier to distinguish a hormetic (biphasic) rather than a threshold model from an LNT model. Secondly, the hormetic model had a threshold component and the principal goal of industry was to establish a threshold and not, as some would believe, to argue for public health benefits due to a hormetic response from low-dose carcinogen exposure, which would have been politically problematic for industry, even if true.

In August 1985, the electrical power industry from the US and Japan held a conference to explore the concept of hormesis and published the proceedings in the peer-reviewed journal *Health Physics* in 1987, essentially igniting the "hormesis debate" as relates to cancer risk assessment. The proceedings were followed two years later by a debate about radiation hormesis in the journal *Science* by leaders of that initial conference [140,141]. Although the issue of hormesis had resurfaced during the 1980's, it was a modest initial revival as reflected by citations in the Web of Science, which averaged less than 10 per year during that first half of that decade.

9.2. Biphasic dose responses-multidisciplinary

Despite the slow scientific rediscovery of the hormesis concept during the 1980's, there were other developments suggesting a convergence on the topic of biphasic dose responses. None was related to policies or specific actions by regulatory agencies. Reports from multiple scientific fields independently demonstrated the occurrence of hormetic-like biphasic dose-response relationships. For example, Szabadi et al. [142, 143] summarized the occurrence of biphasic dose responses as far back as 1906, starting with research of the 1936 Noble Prize winner Henry Dale (1875–1968), proposing a receptor-based mechanistic model for biphasic dose responses. The Szabadi papers [142,143] generated supportive commentaries and related references [144–148], providing a foundation for further research developments. In a similar fashion, numerous epidemiological studies showed U-shaped dose responses for various types of medical and public health outcomes [149], while Stebbing [150–153] extended hormetic observations to environmental toxins. During this period, researchers in genetic toxicology reported that low doses of chemical mutagens could induce DNA repair processes which would protect against subsequently more massive exposures to the same or different mutagenic agents [150]. By 1984, adaptive responses to ionizing radiation had been reported [154]. In 1986, the concept of preconditioning generated much scientific excitement when a short-term/low-dose hypoxic stress was administered prior to the induction of a massive myocardial infarction and reduced cardiac damage by about 70–80% [155]. These findings described the adaptive response and were soon generalized to other cell types, organs and biological models. The biphasic shape of the dose response in each of these general areas defined the hormetic dose response [71,72]. In parallel with these emerging findings was the rapid development of *in vitro* methods that created the opportunity to assess more doses/concentrations per experiment. The adoption of *in vitro* testing and its high throughput dimension markedly increased the number of examples of hormetic dose responses in the biomedical literature.

9.3. Hormesis database

Since the mid-1990s, there has been a progressive increase in the reporting of hormetic dose responses in the toxicological and biomedical literature. The hormetic response is common as well as very general, being independent of biological model, endpoint, inducing agent, and the potency of the inducing agent. The hormetic dose response displays specific quantitative features, with the maximum response being about 30–60% greater than the concurrent control group (Fig. 3) [156–159].

Demonstrating a hormetic dose response requires greater sample size/statistical power in the low dose zone along with careful consideration of dose spacing selection with more doses below the threshold along with a heightened need to replicate findings. Given the modest nature of the low-dose stimulation, understanding the historical variation in control groups is critical.

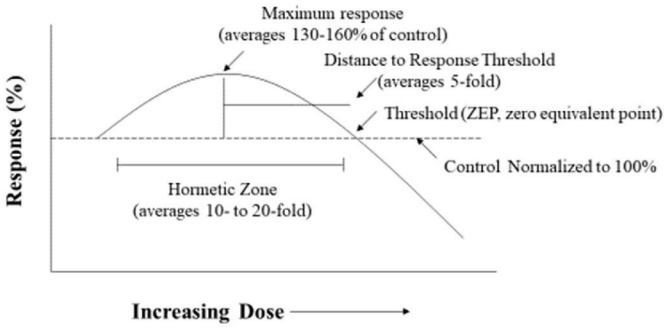

Fig. 3. Dose-response curve depicting the quantitative feature of hormesis (Source: Based on Calabrese [160]).

9.4. Defining hormesis

The low-dose stimulatory hormetic dose response is inherently neither beneficial nor harmful [161–164], being dependent upon the biological context of the effects. When the low-dose stimulation results in enhancing longevity, greater disease resistance/reduced disease incidence, improved memory, greater bone strength, and other comparable apparently desirable responses, it has generally been viewed as "beneficial". When the responses reflect the enlargement of the prostate gland, the enhanced proliferation of tumor cells or other undesirable responses, it has been viewed as potentially harmful [163,165]. In some cases, the clinical or public health implications of low-dose stimulations (responses) may not be large enough to be practically significant. In such cases the clinical or societal implications may be uncertain.

9.5. Hormesis frequency

Using rigorous *a priori* entry and evaluative criteria the frequency of hormesis in the toxicology and pharmacology literature approached 40% [166–170]. This observation was extended by several large-scale evaluations [171–173].

9.6. Validation of dose-response models

These findings on the frequency of hormesis raised the question as to how the toxicology and pharmacology communities had validated the threshold dose-response model during the 20th century. Detailed searches of the literature failed to reveal published attempts to test the general validity of the predictions of the threshold dose-response model in the below threshold zone, suggesting a major failing of the scientific and regulatory communities.

When the concept of validation was then applied to the three dominant dose-response models (i.e., threshold, LNT and hormetic) using multiple independent large data sets, the only model that was consistent in accurately predicting responses in the low-dose zone was the hormetic model. The threshold model consistently failed to accurately predict responses across the entire spectrum of agents tested [173]. Inaccurate predictions were most frequent for the LNT model, making LNT the most unreliable. Thus, multiple decades after their acceptance, critical inadequacies were uncovered for the two key dose-response models (i.e., threshold and LNT) used by regulatory agencies for risk-based assessments.

From an historical perspective, a hazard assessment process was created based on the assumption that the threshold dose-response model provided accurate estimates for responses below the threshold. This hazard assessment testing protocol determined how chemicals would be tested and evaluated, including the animal models selected, their background disease incidence, the number and the spacing of doses and the dose selection strategy. In fact, as a result of accepting the threshold dose response as the model to estimate responses in the low-dose zone, toxicology would become a discipline using only a few very high doses in the chronic bioassay. These decisions, based on the threshold model, would ironically affect the cancer risk assessment process from the late 1970s onward.

The hazard assessment process evaluates possible carcinogenicity at the highest dose which does not exceed the maximal tolerable dose (MTD). There was no interest in obtaining information on the entire dose-response continuum since it was assumed that only random bounce or noise would be observed for responses to doses below an estimated threshold. This type of thinking reinforced the assumption that the hormetic dose response did not exist.

There are multiple flaws in this hazard-assessment process. If the high dose were toxic and could not be used, then the study would be dependent upon only one or two doses. Since the second dose was 50% of the high dose, it was also at risk for exceeding the MTD. Regardless of whether both doses provided valid data, concerns would still remain over the response at lower doses and whether a threshold reasonably well characterized the most sensitive endpoint.

The background tumor/disease incidence could affect the selection of the animal model. In the case of the hormetic dose response model, it would be necessary to test the predictive capacity of the model by ensuring that the tumor incidence of the control group and other disease endpoints would be such that one could detect a decrease in the incidence if hormesis were present. If the background (i.e., control group) incidence was negligible then it would be impossible to detect the presence or absence of the hormetic dose response. The background disease incidence was not of theoretical concern for the threshold model.

In summary, the selection of the threshold model by the regulatory community was never validated throughout the 20th century. It was simply adopted by regulatory agencies such as EPA, OHSA, FDA and all state relevant regulatory agencies, guiding the testing of all chemicals and drugs for safety.

9.7. Failed attempts to validate the LNT model

9.7.1. The mega-mouse study

In contrast to the failure of governments to validate the threshold dose-response model, the US FDA attempted to validate the LNT dose response model for chemical carcinogens. The strategy involved the selection of a well-studied chemical carcinogen (i.e., 2-acetylaminofluorene – 2-AAF) and the evaluation of the LNT model using 24,000 mice. The results were extensively reported [174–176] and assessed by an expert panel of the US Society of Toxicology (SOT) [177]. Since the study was only able to assess tumor incidence to the level of one percent, it became known as the ED01 study. It was not possible, therefore, to practically test the LNT model for cancer risk assessment at the levels of risk that regulatory agencies need to estimate, that is, in a range from one in 10^4 to one in 10^6. As a result, no other rodent-based mega-mouse cancer study has been subsequently undertaken to assess/validate cancer-risk predictions in the low-risk zone.

Despite these limitations, the SOT committee developed a dose-related time to tumor model for the ED01 study. 2-AAF induced a J-shaped dose response for bladder cancer, a finding that was consistent in all six rooms in which the large numbers of mice were maintained (Fig. 4). There was both a threshold and a significant reduction in risk at doses below the threshold. Without using the term, the SOT panel concluded that the experimental findings demonstrated a hormetic dose response.

In an attempt to validate the LNT model, Japanese researchers

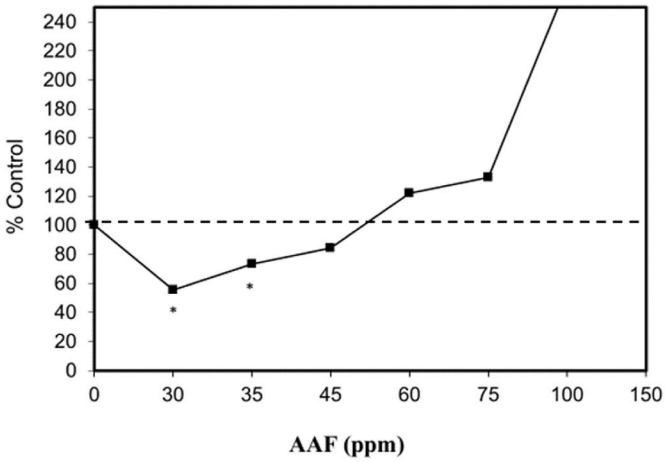

Fig. 4. Bladder tumor incidence adjusted for time in ED01 mega-mouse study: Hormetic dose response relationship (Source: Based on Bruce et al. [177]).

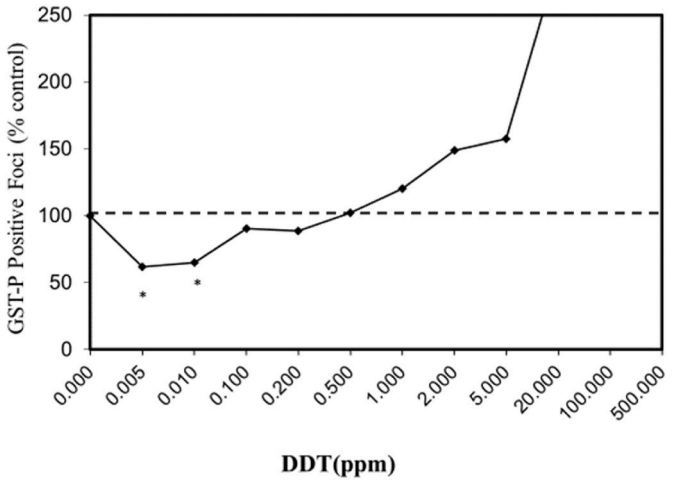

Fig. 5. Effect of DDT on liver foci formation in male F344 rats: Hormetic dose response relationship (Source: Based on Sukata et al. [178]).

assessed the dose response of the liver carcinogen DDT using liver foci as the endpoint [178] (Fig. 5). The study findings contradicted the LNT and supported the hormetic model.

10. Mutagenicity and low-dose linearity

William Russell's long-term research on the effects of ionizing radiation on mutation frequency in mouse germ cells, which involved the use of over two million animals, provided a significant opportunity to evaluate the validity of the LNT model. Russell's research showed that dose rate rather than total dose determines mutation risk, that the dose-rate data revealed the existence of DNA repair processes, and that a threshold response in oocytes occurred at 27,000 times higher than background ionizing-radiation exposures. Since these findings were with a mammalian model, his data become far more relevant than earlier data with fruit flies [26,81].

While these research developments had challenged the validity of the LNT dose-response model of which Muller was the chief proponent, Russell was reluctant to actively press the issue until after the death of Muller (April 1967). Russell then openly challenged the mantra of radiation geneticists that the mutational dose response to ionizing radiation was irreparable, cumulative, and linear.

In a 1970 presentation at the 14th International Congress of Radiation Research at Evian, France, Russell [179] recalled that the original estimates of genetic risk (which were made by the BEAR I Genetics Panel [54]) for radiation were based on two major assumptions: (1) the frequencies of radiation-induced gene mutations in the fruit fly have extrapolative relevance to humans and (2) results from radiation experiments on fruit fly spermatozoa illustrate general principles of radiation genetics and thus can be applied to humans.

What followed from these two assumptions was a series of fundamental risk-assessment tenets upon which genetic and cancer risk assessments were based. According to Russell [180] they included: 1) "Gene mutation rate is directly proportional to radiation dose; 2) Gene mutation rate is independent of radiation dose rate; 3) Gene mutation rate is independent of dose fractionation; 4) There is no repair of gene mutational damage; 5) There is no threshold below which no genetic damage occurs; and 6) There is no recovery from mutation with time after irradiation". After more than two decades of conducting genetics research on mice at Oak Ridge National Laboratory, Russell had evaluated the effects of ionizing radiation in the largest ever progressive/cumulative mammalian study in the history of science. From this experience, Russell concluded that, regarding the two major assumptions, "the first assumption is probably not valid, that the second is definitely incorrect, and that each one of the six 'general' principles does not apply to mouse spermatogonia and/or oocytes." During his presentation, Russell offered substantial scientific evidence supporting these conclusions, challenging the validity of the LNT model.

Although Muller was quick to adopt a strong belief in the LNT concept (i.e., Proportionality Rule of 1930), his Nobel Prize research was performed using dose rate that were a massive 95 million fold greater than background during the exposure period [13,180]. That Muller could/would extrapolate this dose rate range over eight orders of magnitude and not be challenged for his predictive exuberance suggests how high his standing was in the scientific community. Subsequent studies by Giles [181] in *Tradescentia* used a dose rate 10,000-fold greater than background and failed to detect any effect after assessing about 3000 chromosomes, indicating a threshold dose response.

11. Background radiation and mutation

Mammalian cells display a steady state level of DNA damage. This damage condition reflects a low error frequency of 10^{-10} mistakes per base pair each replication cycle that may be itemized by mistakes at initial base paring, proofreading and mismatch repair. DNA damage occurs principally from normal oxygen metabolism via the action of reactive oxygen species (ROS), along with deficiencies in selective nutrients such as folate, and other endogenous and environmental toxins which can add more multiple millions of genetic damage events per cell per day. The quantity of DNA damage due to endogenous ROS when adjusted for repair half-life over 24 h/day, yields of about 10^6 DNA oxidamages occur per cell/day. Based on studies of DNA oxidative genetic damage, and their half-lives for tissue repair, the probability of a single DNA nucleotide out of the total of about 6×10^9/cell being endogenously damaged/day is estimated to be $10^6/6 \times 10^9$ equals 1.5×10^{-4} (damaged nucleotides: 1.5/10,000 total pool of nucleotides). The daily production of endogenous DNA alterations is estimated to exceed that produced by low linear energy transfer (LET) background radiation (1 mGy-annual) by about 200,000,000 fold/cell/day. According to Pollycove and Feinendegen [16], this massive ratio reveals that the system that controls DNA damage and sustains cellular integrity evolved in response to endogenous damage rather than from background radiation damage.

A further consideration of low-LET radiation induced damage indicates that about 2×10^{-2} of the damage is comprised of double strand breaks (DSBs) [16]. DSBs occur due to the effect of ionization clusters and are a measure of potentially serious damage to mammalian cells in comparison to single strand breaks (SSB and base changes) which are more efficiently repaired. In terms of genetic damage to a cell from low LET radiation, the amount of DSBs is about 1/50th of the total radiation-induced genetic alterations/cell/day or about 1×10^{-4}/cell/day from background radiation.

While there is a clustered distribution of ionization and initial ROS from ionizing radiation (IR), endogenously produced ROS is quite widespread. In the case of endogenous ROS, about 2–3% of metabolized O_2 is converted to ROS and leaks out of the mitochondria yielding 10^{10} cytoplasmic ROS molecules/cell/day. This leads to the average of the 10^6 DNA damage events/cell/day for ROS along with micronutrient and environmental toxins as causes of mutation [16].

The rate of DSBs/cell/day produced by endogenous sources compared to those produced by background radiation of 1 mGy/year is about $10^{-1}/10^{-4}$ or approximately 1000 (10^3)/1. Thus, the rate of all DNA alterations produced/cell from non-radiation sources compared to those produced by background radiation is about 2×10^8. However, DSBs only make up only 10^{-7} of the endogenous DNA alterations, about 2×10^{-2} of the background DNA alterations [16]. Thus, background radiation is about 10^5 more efficient in producing DSBs than ROS from endogenous metabolism. Even though the radiation is far more efficient in producing DSBs than endogenous metabolism, the amount of ROS generated from endogenous metabolism is so much greater than the damage from background radiation that, endogenous background ROS

still generates 1000 times more DSBs than background radiation per day.

With such a massive amount of ROS being produced/released to each cell/day an anti-mutagenic DNA damage control biosystem evolved that efficiently reduces the number of events/cell/day. According to Pollycove and Feinendegen [16] this anti-oxidant system reduces the genetic damage events by a factor of 1000 to 10^6. DNA repair processes then reduce these cellular events by about 10^4, leading to a 10^2, which are deemed as persistent DNA alterations. However, other activities, including apoptosis, immune response, and other factors bring the total one mutation/cell/day, totaling about 400 mutations that accumulate per cell per year.

That background radiation induces genetic damage that is strikingly less than produced by endogenous metabolism was recognized nearly a century ago by Muller and Mott-Smith [180] when they estimated that background radiation could only account for 1/1,300th of the genetic changes in the control group of his Nobel prize research. Thus, it was not possible to even detect the influence of background radiation within highly controlled experimental systems and, of course, within far more variable epidemiological studies, despite the longer human reproductive period/lifespan.

Building upon this perspective further, Pollycove and Feinendegen [16] estimated what might happen if the background dose increased by a factor of 10 such that the increase in genetic damage hits per cell per day increased from 1 to 10. They reported that low doses of ionizing radiation would induce enzymatic DNA repair mechanisms leading to significantly decreased spontaneous malignant transformation in cell culture (including possible bystander induced genetic damage) by about 70% (See Azzam et al. [182]; Redpath et al. [183]). The decrease in damage occurred over a 100 fold dose range that encompassed exposures ranging from normal biological background to an exposure 100 times greater (100 mGy). They indicated that these laboratory estimates were supported by numerous population studies from high natural background radiated areas in India, Iran, China and elsewhere as well as in the largest cohort of occupational nuclear workers and various medical cohorts (see Pollycove and Feinendegen [16], page 300 for references). The low dose stimulation of the entire DNA damage control system (i.e., anti-inflammatory system, DNA repair, apoptosis, etc.) would most likely act predominantly on the non-radiation-induced DNA damage over a period of several hours to weeks. This hormetic response is generally restricted to low doses, declining at higher doses.

Of clear relevance to the LNT issue is that cells have an evolutionary-based DNA control system that acts, in effect, to ensure the integrity of genetic information, acting as an anti-mutation biosystem. Thus, it is expected that gene mutations that are induced by endogenous ROS would be reduced by low doses of low-LET radiation via induction of the adaptive response. These findings indicate that hormetic and possibly threshold-like responses could likely predominate in such situations, with LNT being highly unlikely.

12. Bad Luck mutation hypothesis

In 2015, Tomasetti and Vogelstein [184] published an article in *Science* concluding that random mutations that occur during DNA replication in normal, noncancerous stem cells account for about 70% of human cancers. This provided the basis of their "Bad Luck" cancer hypothesis, with the implication that most cancers have a strong element of chance or randomness to their occurrence. This view challenges some longstanding beliefs that most cancers arise due to exposure to naturally occurring or manmade carcinogens. The issues raised not only speak to risks at the individual level but also how risks may be distributed across tissues. This hypothesis is founded on the basis of linking tumor initiation (i.e., random mutation) to a promotional (stem cell division) stage of carcinogenesis. These developments are likely to be linked with other tumor-promoting factors that can accelerate or slow the rate of tumor development. A similar concept was addressed earlier when discussing the latency concept and how tumor-promoting factors can significantly affect the growth of a tumor [138]. Thus, the Tomasetti and Vogelstein hypothesis is a complementary one to the tumor-latency proposal, but one that was framed somewhat differently. It demonstrates the significance of a specific type of endogenous promoting stimuli (i.e., stem cell proliferation) and how this may be an important driver in a broad spectrum of cancer risks. There is an element of randomness involved concerning when and what tissues develop cancer, as they suggest, since each cell experiences large numbers of gene mutations per day from mostly endogenous metabolism.

13. LNT historical evaluation: how LNT became institutionalized by regulatory agencies

13.1. Ionizing radiation

13.1.1. The Muller/NCRPM connection

Hermann J. Muller [10] reported that high doses of X-rays induced gene mutations in the germ cells of male fruit flies. Based on these and follow up investigations [185], Muller argued that the gene mutation rate would be proportional to the dose of energy adsorbed, implying no threshold. He created a concept called the Proportionality Rule in 1930, the forerunner to the term linear non-threshold [26].

With respect to ionizing radiation, the idea of a threshold dose response was first proposed in the mid-1920s based on occupational exposure concerns [25,186]. The threshold concept assumed that workers could tolerate a certain amount of exposure without significant health concerns. It was generally accepted, especially by the medical community, that a threshold dose needed to be exceeded for harm to occur. However, Muller's Proportionality Rule for the occurrence of genetic damage challenged the notion of a threshold.

The first potential "regulatory' impact of Muller's findings challenging the threshold model occurred in 1935 within the American X-ray and Radium Protection Committee, later (1946) called the National Committee on Radiation Protection and Measurement (NCRPM) [46]. At this time an unidentified committee member recommended that the tolerance dose be reduced from 0.1 to 0.05 r/day based on Muller's gene mutation findings. However, the Committee was uncertain how to extrapolate Muller's fruit fly results to humans. Nonetheless, these gene mutational concerns remained as the Committee addressed reproductive safety within an occupational setting and for patients. Over the next several years, this Committee's discussions demonstrated how Muller's mutational findings came to progressively challenge the threshold concept for assessing the risk of ionizing radiation. At the December 1938 meeting of the Committee, a proposal was offered to modify the definition of tolerance dose as follows: "The generally accepted tolerance dosage is taken as 10^{-5} r/sec for a 7 h day. Geneticists on the Committee pointed out that because of the cumulative effect of X-rays the tolerance dose should not exceed 10^{-6} r/sec (Whittemore [46], see footnote 300)." By the December 1940 meeting, the Committee reported that the rationale for lowering the tolerance dose was due to concerns with mutational effects (Whittemore [46], see footnote 305). This recommendation concerned the influential Failla, who argued that changing to a mutational endpoint would create substantial uncertainty since there may not be a safe level or threshold for genetic mutations. However, the available data were still not considered convincing.

The Committee struggled to resolve the threshold vs linearity dilemma. Although it believed the term tolerance dose referred to a dose that could be tolerated without any biologically significant damage, this was not the case with genetic damage that was believed to be irreversible, cumulative and harmful based on Muller's interpretations. The dose response change from threshold to linearity led to the novel concept called "permissible exposure", that is, an acceptable dose that could still injure and was therefore not considered "safe". "Permissible exposure" became the means by which the term "acceptable risk" became operational. The Committee adopted "permissible exposure" as

both a compromise and a practical decision that enabled the exposure standard to remain unchanged. This satisfied supporters of the threshold position even as they yielded on the principle. The dose response for ionizing radiation would therefore change from a tolerance position that was based on the threshold model to a linear one based on gene mutations. However, despite the years of debate and the change in terminology and concept, this alteration of critical policy was never officially promulgated or published. As a result, this failure led to new controversy immediately after World War II [46].

In December of 1946, Muller received the Nobel Prize and shortly thereafter joined the NCRPM, which was still chaired by Failla. In their 1947 draft report [46], the NCRPM finally relinquished on the concept of tolerance dose due to the Committee's consensus belief that ionizing radiation produced cumulative amounts of genetic damage and had no threshold. Once again, organizational inertia and personal disputes intervened and the NCRPM recommended changes failed to be published in 1948. This inability to finalize and publish policy continued for six years, making the actions of the NCRPM somewhat skeptical and difficult to predict.

On September 24, 1954 the NCRPM [187] published "Permissible Dose from External Sources of Ionizing Radiation (National Bureau of Standards Handbook 59). This Committee was composed of eight members with Hermann Muller and Curt Stern part of the group. This major publication, even though obscure with respect to the scientific community and general public, was highly significant since it represents the transition from threshold to LNT for genetic risk and for the adoption of the Precautionary Principle. On page 17, the committee writes: "It has been shown experimentally that genetic changes can be produced with low doses of radiation. The frequency of occurrence increases linearly with the dose in the case of gene mutation …" On page 19, it is stated: "Most of the information on these effects has been obtained from animal experiments but it may be taken for granted that the same effects occur in man …"

In the Committee statement on Acceptable Risk, the LNT concept was clearly adopted with the statement "that any predicted limit of exposure that may be set up today will involve some risk of possible harm." This position then led the Committee to adopt the concept of "acceptable risk" since there was no threshold. The Committee then went beyond the consideration of genetic risk, extending their concerns to cancer, specifically focusing on leukemia. The Committee also set the stage for the Precautionary Principle, making the pitch that adverse effects seen at high doses in animal studies can create a reasonable expectation of occurrence in humans, even with "small daily doses."

One can see the influence of Muller and Stern as they teamed up to direct the focus of the Committee, relying on the disputed Stern studies with Drosophila during the Manhattan Project to compel the adoption of LNT. It should be noted that when the BEAR I Genetics Panel was created the very next year, Muller and Stern were again given another chance to influence the LNT decision. In this case, Muller accepted while Stern declined due to exhaustion from recent travels, only to be replaced by another leading radiation geneticist who supported LNT.

13.2. BEAR I Genetics Panel-1956

Around this time, a newly formed NAS/National Research Council (NRC) committee (the BEAR I Committee, Genetics Panel) soon overshadowed the activities of the NCRPM and, interestingly, also came to be dominated by Muller and other radiation geneticists who were committed to the LNT model. Like Muller, other scientists possessed clear ideological perspectives and served on multiple national and international committees addressing the same dose-response issues on policy [25], in effect permitting them more than one bite at the proverbial apple of radiation risk assessment.

In its 1956 publication, the NAS BEAR I Genetics Panel [55] recommended adopting low-dose linearity for the induction of germ cell mutations by ionizing radiation. This Genetics Panel became the vehicle that would change risk assessment policy. The 1956 report was widely distributed, received substantial media attention, enhanced adoption of the LNT and stoked radiophobia in the media and general public [26].

The transition from threshold to linearity had taken about three decades, originating with Muller's 1927 publication [10]. During an oral history (Taylor, Oral history: www.aip.org/history-August 11, 1990), the longtime chairman of the NCRP, Lauristan Taylor, noted that his committee looked to the NAS BEAR I Genetics Panel for guidance on this matter. Within about 18 months of the 1956 NAS report, the NCRP expanded the concept of linearity from germ cell to somatic cell mutation, thereby affecting cancer risk assessment [25]. Later other national and international committees would adopt this recommendation with significant long-term implications and consequences.

13.3. BEAR Genetics Panel-1960

Although the 1956 BEAR I Genetics Panel did not formally address the issue of somatic effects, the 1960 BEAR Genetics Panel did, stating that it "does not consider it justifiable to predict human tumor incidence from small radiation doses based on extrapolation from the observed incidences following high dosage" [188]. Despite its dramatic policy significance, this statement of the BEAR 1960 Genetics Panel received no discussion in the general scientific community nor has it been cited in debating threshold vs LNT [34]. The 1972 BEIR I Genetics Subcommittee likewise ignored the report by the 1960 BEAR Genetics Panel and only referenced the LNT recommendation report by the 1956 BEAR Genetics Panel. The BEIR I [65] Committee claimed to have re-affirmed the linearity position of the BEAR I Genetics Panel [55] (which applied only to mature germ cells and not to somatic cells) and continued to apply LNT to cancer risk assessment.

The 1960 BEAR Genetics Panel also acknowledged that the number of mutations in mouse spermatogonia and oocytes were less when cells were exposed to X-rays at a lower dose rate than when cells were exposed to a higher dose rate even though the total dose was identical under both exposure scenarios [188]. This observation was based on findings in mice by Russell et al. of December 19, 1958 [66], as published in *Science*. Ten years earlier, this was also the principal finding of Caspari and Stern [38] in a study with Drosophila sperm that had caused considerable scientific confusion due to multiple deceptions by Stern and Muller as they attempted to deflect and diminish Caspari's findings, which had challenged the LNT concept [28,31,189,190]. The 1960 NAS BEAR Genetics Panel acknowledged that the mantra of a cumulative, irreversible and linear radiation-induced mutational response was now challenged by Russell's dose-rate studies with mice. In fact, the Panel also wrote that the linear risk-assessment model of the 1956 NAS Panel did not apply to mouse spermatogonia and oocytes, "which are the most important cell stages as far as human hazards are concerned" [188].

These historical actions need to be viewed for how they have affected scientific beliefs and public policy in the dose-response and risk-assessment domains [186]. The NCRP gave the impression of deferring its position to the 1956 BEAR I Genetics Panel; yet it developed a position on somatic cells that was not formally addressed in a direct policy sense by this NAS/BEAR I Genetics Panel, which was actually in apparent conceptual disagreement with it. Ironically, and as noted above, the 1960 BEAR Genetics Panel explicitly did address a position on the cancer response in somatic cells and did not support the concept of extrapolating from high to low doses for cancer risk. This position contrasts with the actions of the NCRP to recommend LNT for cancer risk assessment based on a Precautionary Principle. However, it was the 1956 report of the NAS BEAR I Genetics Panel that received enormous publicity within the scientific community and the popular press while the 1960 NAS BEAR Genetics Panel's report was overlooked (i.e., not published in *Science* journal or actively promoted) and lacked any notable impact as a result.

13.4. The Federal Radiation Council (FRC)

In 1959 President Eisenhower and the US Congress created the FRC. The new Council was created to advise the President, Congress and federal agencies on health risks from exposures to ionizing radiation. In creating the FRC the President was forced to publicly acknowledge that he had lost confidence in the Atomic Energy Commission (AEC) to guide the country on matters related to health and safety for ionizing radiation. Ever since the US started to conduct above ground nuclear tests in Nevada, the AEC had become a target of criticisms for downplaying genetic and cancer risks. The issue became heightened and focused in the aftermath of the fallout from the H-bomb testing on the Bikini Atoll in March 1954. The director of the AEC, Lewis Strauss, became the target of serious criticism by highly prominent members of the US academic community, especially the radiation genetics community. While Strauss would argue that the fallout exposures were quite low, far below any harmful threshold, the genetics community was strongly opposed to the AEC position, claiming that it was socially and scientifically irresponsible. The challenge to the AEC by the academic community was strident and at the highest levels, involving multiple Nobel Prize recipients such as Hermann Muller and Linus Pauling. However, this only got worse for the AEC when the US NAS BEAR I Genetics Panel emasculated it with their highly publicized report claiming that there was no safe level of exposure to ionizing radiation and that continued above ground testing would cause increases in a broad spectrum of birth defects, leukemia and other cancers. Within the context of this mounting criticism and societal health concerns, the President eventually came to the conclusion that the AEC had lost credibility, both politically and with the US population. The President acted to remove radiation health risk assessment from the AEC and transfer it to the newly created FRC. This structural change would become a critically strategic repositioning whose effects are still being felt within Society. In a functional sense the FRC that Eisenhower created would become very reliant on the recommendations of the NCRP for radiation risk assessment. This would prove to be a major victory for the radiation genetics community as lead by Muller, Stern and their next generation such as James Crow since they guided the NCRP for the 15 years. Thus, the BEAR I Genetics Panel not only published a far-reaching paper in *Science* in June 1956 [54] making the case for the LNT. Key members of this Panel would display a highly influential role within the NCRP, which would then be the principal advisor for the FRC. For the radiation genetics community, it was like a dream come true. For example, when the FRC issued its 1962 report on the health implications of fallout from nuclear weapons, they acknowledged the guidance of seven scientists, including James Crow, James Néel, Bill Russell, and Howard Andrews, all members of the BEAR I Genetics Panel [190]. In effect, the FRC became the voice of the LNT supporting radiation genetics community. It represents a remarkable scientific, political and institutional revolution. This situation became even more significant when President Nixon created the US EPA in 1970. In this process Nixon eliminated the FRC, transferring their function to the US EPA for radiation health evaluation. Thus, when the US EPA accepted the LNT recommendation of the NAS/NRC BEIR 1972 panel [65], it was simply a continuation of the FRC accepting the guidance of the ideologically derived leadership of the radiation genetics community.

13.5. The Delaney Amendment

While the 1956 NAS BEAR I Genetics Panel and other major advisory committees were adopting the LNT perspective, an important independent action in the US Congress reached a similar conclusion, also with major implications. That is, the Delaney "Amendment", which was appended to the Food Additives Amendment, became law on April 26, 1958, declaring that no additive would be considered if it induced cancer in animals or humans. The Delaney Clause was legislatively placed into the Color Additives Amendment of 1960 in response to the highly publicized cranberry crisis of 1959 [191].

The Delaney Clause had its origins at a meeting of the IUAC in Rome in 1954 [75]. Participants at this meeting firmly believed that a single mutation was an irreparable, and thus irreversible, event that initiated an irreversible process that in turn led to an irreversible outcome, the development of a cancerous tumor. The IUAC unsurprisingly decided that small doses of irreversibly acting agents were dangerous. At the time of this meeting, the only viable model that could explain the irreversible effects of mutagens/carcinogens was the LNT single-hit model of Muller and his colleagues. Two years later in 1956, this concern became codified in a recommendation for a proposed rule on carcinogenic food additives that came from the International Conference Against Cancer.[14] It was this recommendation that provided the basis for the Delaney provision on zero carcinogen exposure as it precluded the need for deriving tolerances (e.g., safe levels) for carcinogenic agents. The fact that the wife of one of Representative Delaney's close aides was diagnosed with cancer during the proceedings might have helped persuade Delaney to create and implement such a conservative anti-cancer clause [192].

The FDA would subsequently modify the Delaney Clause, clarifying the issue of *de minimus* risks. These are risks that are too low to be accurately measured and below practical concern. Such a modification permitted FDA to allow carcinogens to be added to food if the estimated risk was lower than a calculated risk (e.g., $\leq 10^{-6}$ per 70 year lifetime) said to be *"de minimus"*, based on animal extrapolation and statistical modeling.

13.6. The safe drinking water act - 1974

The next important challenge for the threshold dose-response model occurred about two decades later in 1977 when the NAS Safe Drinking Water Committee (SDWC) [193] recommended that the EPA adopt the LNT based on the NAS BEIR I report and apply it to the assessment of cancer risks from exposures to chemical carcinogens and ionizing radiation [65]. From the late 1950s to the early 1970s, the debate focused on the linearity versus threshold policy decisions needed for assessing cancer risks from exposures to ionizing radiation. The NCRPM, as discussed above, first recommended the switch from threshold to linearity and mediated an important compromise [194], getting the linearity and threshold advocates to admit that the data supporting their respective positions were not convincing. As a result, the Committee concluded that it was not possible to know the precise nature of the dose response in the low-dose zone. The Committee therefore adopted a conservative position by assuming a LNT dose-response relationship [47,195]. Although this position appeared to represent a compromise, it was a victory for the "precautionary principle", reminiscent of the compromise in the 1940s concerning tolerance versus permissible dose. However, in that earlier situation, the outcome was considerably different. Permissible dose was accepted as scientifically more plausible even though the threshold dose response would govern the regulation. Approximately 15 years later, the concept of linearity had lost much scientific support (e.g., Russell's dose-rate study) but its underlying philosophical foundation (i.e., the precautionary principle) was adopted by the NCRPM. Other major advisory organizations would also confront this problem. For instance, the NAS BEAR Genetics and Medical/Pathologic Panels of 1960 were non-committal on scientific grounds while the US FRC [190] outright accepted linearity and its reliance on the precautionary principle.

This somewhat chaotic struggle between the LNT and threshold models would continue among various committees and their members

[14] The key phrasing statement for the 1956 Rome meeting of the IUAC was adopted by representative Delaney: "As a basis for active cancer prevention the proper authorities of various countries promulgate and enact adequate rules and regulations prohibiting the addition to food of any substances having potential carcinogenicity."

for the next decade. The precautionary perspective would re-emerge with the next NAS committee (now called BEIR I, 1972) [65], a perspective that markedly affected major policy recommendations of the 1977 NAS SDWC [193]. The SDWC also relied upon a series of studies they claimed supported the scientific foundations of low-dose linearity [196–199].

The EPA accepted the 1977 recommendations of the SDWC and two years later applied the LNT model to the risk assessment of trihalomethanes (THMs) in drinking water; LNT has continued to be applied to dozens of other chemical carcinogens up to the present day. The theoretical basis of this challenge to the threshold dose-response model for chemical carcinogens was founded upon eight guiding principles articulated by the NAS SDWC [193]:

1. Only one or two changes in a cell are needed to transform it to cancer.
2. Human population heterogeneity should drive the assessment of cancer risk as some people will be at greater risk. Such heterogeneity suggests there is no population-based threshold.
3. A transformed cell will be irreversibly propagated.
4. If the mechanism involves mutation, there is no threshold; in fact, if there were no information on mechanism and cancer occurred, mutation should be assumed.
5. A single molecule or a few molecules can cause a mutation. Therefore, linearity at low dose can be assumed.
6. The tumor response to a carcinogen was assumed to be additive to background, acting via the same mechanism as spontaneous cancers. This would ensure a linearity conclusion even in the presence of a threshold dose response.
7. Available data on radiation-induced mutagenicity demonstrated that it was linear at relatively "low" doses.
8. Since chemical carcinogens act like ionizing radiation, low dose linearity applies to chemicals as well.

The SDWC neither documented possible opposing arguments to low-dose linearity nor cited various weaknesses in the so-called eight principles. Furthermore, the Committee failed to rank the above-cited principles according to their degrees of scientific confidence. The SDWC should have distinguished those principles that were supported by credible scientific data from those supported as being philosophically more protectionist. In addition, some of the key references used by the 1977 SDWC provided data and/or conclusions that directly contradicted principles #1, #3, #4, and #5. For example, Nordling [199], Muller [197], and Iverson and Arley [198] argued that the occurrence of multiple successive mutations in the same cell (i.e., up to seven mutations) would be needed to cause human cancer, thereby challenging the credibility of some of the Committee's principles.

Although each of the eight "guiding principles" provided support to the EPA and enabled it to adopt low-dose linearity in assessing the health risk of carcinogens, data generated during the intervening four decades have not validated any of these "principles". In fact, quite the opposite, new findings have revealed that those principles are generally scientifically untenable or, from the practical standpoint of detection sensitivity, impossible to assess. For example, that a single point mutation would be sufficient to cause cancer or that the process of carcinogenesis, once initiated, is irreversible, has been repeatedly discredited [99,100,102]. Driver et al. [200] reported a linear dose-response relationship for dimethylnitrosoamine (DMN)-induced adducts and foci in the kidney of male F344 rats. However, after a while, the linear dose-response pattern disappears, revealing a threshold dose response for this genotoxic carcinogen. Such dose-response data, when evaluated periodically over time, reveal a multistage process that involves, for example, the early and rapid repair of carcinogen-induced damage—especially at low doses—and the elimination of initiated and transformed cells, resulting in a regression/suppression and remodeling of the entire carcinogenic process.

14. The limits of epidemiology

14.1. Population-heterogenicity-based LNT

Although each individual of a population may display a threshold response, the population itself may display no threshold due to heterogenicity within the highly outbred and socially/culturally diverse human population. This is principally a theoretical position since the power of epidemiological studies to estimate low risks is usually weak, due to limitations in assessing exposure, genetic variability, and numerous other disease modifying variables.

The scientific foundations of a heterogeneity-based LNT model assumes that there are multiple risk factors affecting susceptibility to toxic substances and that these risk factors are randomly distributed within the population. This would result in a rather broad distribution of susceptibilities with multiple interactive risk factors affecting a progressively smaller proportion of the population who would likely display a greater level of risk and/or susceptibility. When taken to its extreme, individuals with the greater number of overlapping/additive/interactive risk factors would likely be the first to experience adverse effects, including premature death. The heterogenicity-LNT concept also assumes an additive-to-background adverse effect relationship that further supports a linear interpretation.

The evidence to support the heterogenicity-LNT hypothesis is difficult to obtain since epidemiological associations usually are not viewed as causative unless the risks significantly and consistently exceed the control or reference population. In the area of toxic torts, formal judicial guidance reflects this perspective, with judges being guided not to accept a causal relationship unless the relative risk for the alleged exposure equals or exceeds a factor of 2 [201]. If, therefore, the heterogeneity-LNT dose-response model were to be accepted, it would come into conflict with current standards for epidemiological guidance for toxic torts and risk causality [202].

Factors such as family characteristics, age, gender, stress, diet, prior disease, access to medical treatments, social factors, transgenerational epigenetics, and other influences may affect health outcomes. Given all the possible confounding variables and the extreme difficulty of their quantification or the general inability to control for such differences with surrogate and likewise variable and uncertain parameters (such as socio-economic and other standard population metrics), the capacity to use epidemiological methods to estimate risks to less than the doubling rate is generally beyond the present capacity of this discipline. Thus, the population-based, heterogenicity-LNT model is constrained by the inherent limitations of epidemiological methods. Even with marginal improvements in sensitivity, the capacity for epidemiological methods to address low-dose risks is principally theoretical, model dependent, and frequently without the capability for validation. While this is a powerful argument against the population heterogeneity-LNT hypothesis, it is similar to the argument against the LNT dose-response model that is based on experimental evidence derived from only a few very high doses in the standard chronic bioassay.

Another way to evaluate the population-heterogenicity argument relates to the number of molecules needed to induce toxic/carcinogenic effects. At reasonably safe drinking-water exposures (the EPA standard), most people would consume in the range of 10^{14}-10^{16} molecules/day/person. Even if this were to be lowered by a factor of 10^3 below an already safe level, a vast number of molecules would still be remaining. The heterogenicity argument seen in this context loses relevancy. In fact, followed to its logical progression, Cox [203] suggests that population-based dose-response models for highly improbable events should incorporate the assumption of a minimum tolerance below which no member of the population will respond (i.e., a threshold).

14.2. Ionizing radiation cancer epidemiology displays a highly consistent threshold

Ricci and Tharmalingam [204] analyzed a broad spectrum of epidemiological studies that investigated the effects of radiation on cancer incidence, in atomic bomb survivors, community population-based studies, occupational populations, household radon studies, and leukemia studies. Regardless of the study population, the exposure conditions to ionizing radiation, or the affected populations, the dose responses derived from these multiple meta-type analyses revealed a generally uniform threshold dose response, with adverse effects first observed in the range of 120–150 mSv/day. Findings from 13 epidemiological studies displayed both a clear threshold and a cancer response that became evident at the higher dose levels. The threshold findings occurred whether the data were reported on the basis of total/cumulative dose or via dose rate.

In addition to these investigations, Wakeford et al. [205] assessed the possibility of a causal relationship existing between increased radiation from nuclear fallout and childhood leukemia over nearly a half century, beginning prior to 1950. Children from 0 to 4 and 0–14 years of age were selected due to their assumed enhanced susceptibility and their relatively short latency period for radiation-induced leukemia. Children were exposed for prolonged periods at relatively low dose rates. Childhood leukemia incidence was assessed in 11 large-scale cancer registries over three continents. There was no evidence of an increase in leukemia incidence after the periods of maximum exposure. Data obtained from the testing of atomic bombs indicated that at a low dose rate ionizing radiation did not influence leukemia frequency. These findings indicated both that the standard assessment of childhood leukemia due to radionuclide exposures from fallout is in error and that the very low doses of normal background radiation are essentially undetectable with epidemiological methods. Furthermore, the atomic bomb fallout was considered to be quite similar to radionuclide discharges from large numbers of nuclear facilities. The lack of a peak in the incidence of childhood leukemia following very active above ground nuclear testing provides compelling evidence that the LNT hypothesis is not valid for low-dose radiation.

15. Plotting data: a key element in the threshold vs linearity debate

Substantial discussion has ensued over the plotting/graphing of dose-response relationships in the toxicological literature, especially with respect to tumor incidence. Waddell [206,207] has argued that dose responses should be plotted on logarithmic rather than linear scales. Waddell believed that plotting dose-response relationships on linear scales, as routinely done in toxicological studies, has no scientific basis and, in effect, conceals threshold responses. A study by Rozman et al. [208] informed Waddell's argument and demonstrated that differences in plotting data could affect interpretations of dose-response relationships, especially in the low-dose zone. These authors claimed that the case for low-dose linearity was markedly reinforced by changing from arithmetic to logarithmic plotting of the dose response, as recommended by Gaddum [209] over seven decades ago.

To demonstrate the difference in plotting methodology, Waddell [206] used data from a large cancer bioassay (4000 rats) of the carcinogen nitrosodiethylamine (NDEA). The dose-response data fit a linear dose-response relationship when Waddell plotted NDEA doses on the abscissa in units of mg/kg/day using an arithmetic scale. By contrast, when Waddell plotted NDEA doses on the abscissa in units of molecules/kg/day (ranging from 1 to 10^{23} molecules) using a logarithmic scale [208], he observed a clear threshold effect. The difference between the arithmetic (mg/kg/day) and the logarithmic (molecules/kg/day) plots were quite remarkable. Notably, the cancer risk of a rat appeared to approach background or spontaneous tumor incidence in the arithmetic plot at "zero" exposure to NDEA (indicative of a linear response) and in the logarithmic plot at a decidedly "non-zero" exposure of 10^{17} NDEA molecules (one hundred thousand trillion molecules per day) (indicative of a threshold response). The threshold response represented a general pattern for numerous positive cancer bioassays. Waddell [210] plotted dose responses for 50 chemical carcinogens and revealed that the estimated "zero" response (i.e., that which was equivalent to the spontaneous background response) approximated 10^{17} molecules/animal/day.

The assessment of Waddell [206,207,210–219] generated much interest and debate [220–225]. Crump and Clewell [220] disputed Waddell's interpretation and claimed it to be a visual artifact based on the scale of the plot. According to Andersen et al. [221], no realistic representation of a threshold can be demonstrated even in very large toxicity studies. Andersen et al. [221] argued that the only way to demonstrate a threshold was via a mechanistic assessment of the biological dose-response process. Lutz [222] argued that the log transformation of the dose made it impossible to indicate a background control response because the log of 0 is undefined. While this is an issue, in practice it is commonly dealt with by giving the control group a very small but positive value of no practical consequence.

Waddell's papers became the target of criticism because they challenged a central feature of cancer risk assessment. The plotting of dose-response relationships using the number of molecules as the dose is instructive, providing an improved context for assessing dose-response data. An important value of the Waddell method is that exposure via weight, that is, milligram (mg), is a surrogate for the actual exposure which is the molecule itself, the essential element in the hazard/risk assessment. Such plotting does not exclude other methods of data presentation. Finally, while log plotting of toxicity data is not uncommon, the perspective offered by Waddell is unique as it was used to critique the LNT framework.

16. Guiding rationale for LNT by BEIR

In 1972 the BEIR I Committee/Medicine Committee [65] provided the basis for adoption of the LNT model in cancer risk assessment. In so doing it addressed why the threshold model was not adopted. The BEIR (1972) Committee [65] stated on page 95 that even though a threshold dose for radiation-induced cancer is an appealing notion and some well-known non-linear responses exist in the literature (which they did not cite), "There is no sufficient theory of radiation carcinogenesis from which the concept (of "Threshold Dose") may be deduced and an empirical demonstration has not been made." They further stated that most human data are derived from exposures to high doses and high dose rates. Use of these data requires the extrapolation from very high to essentially a theoretical zero exposure, with the BEIR Report referring to this as an interpolation rather than an extrapolation. Regardless of the terminology, using linear modeling and assuming a Poisson distribution will provide estimates of cancer incidence even at the lowest point on the dose-scale. These low-dose estimates of cancer risk are therefore unavoidable given this stochastic-based process. The BEIR Report [65] emphasized that these estimates are "beyond empirical demonstration" and "it is unlikely that the presence or absence of a true threshold for cancer in human populations can be proved." The Report then states that if the goal of government is to prevent radiation-induced cancer and loss of life then "they [government] must, indeed, be guided by such estimates, and will not rely on notions of a threshold" (page 96). In fact, LNT has been the mantra of radiation geneticists and regulatory agencies over the past five decades. It is somewhat ironic that this mantra is based on a concept that can be neither tested nor disproven by an experimental or epidemiological study even if there were a desire and effort to do so.

It is therefore troubling that the kind of experimental study and empirical evidence that would be needed to justify the acceptance of a threshold model was never a topic of consideration, but instead—as already noted by the BEIR I (1972) report [65]—the failure to conduct such a study and the lack of empirical evidence were actually used to

justify its rejection. Without appropriately designed studies to ferret out evidence specifically targeting the threshold issue, arguments for and against a threshold model become theoretical and subjective. For example, in a series of meta-analyses, Ricci and Tharmalingam [204] argue in favor of a threshold response for radiation-induced cancer and also present some data indicating the possibility of an hormetic response. However, the prevailing biostatistical approach to cancer risk assessment, which was originally adopted far back in the 1940s [226], is to categorically treat such possible hormetic findings as mere variability within the LNT framework. For nearly 50 years, the BEIR committees from I through VII have dogmatically applied a nearly 90-year-old LNT approach that should have been re-evaluated to assimilate massive new dose-response data derived from the application of nearly 90 years of modern biological techniques. This was obviously never done.

Over the past three decades my focus has been to revisit, reanalyze and critique not only the old data originally used in support of the current LNT model but also the newer and more critical data generated post-LNT adoption. This led to an extensive effort to study the hormetic dose response. Because of its unique biphasic characteristics, the hormetic dose-response model contrasts sharply with the LNT as well as threshold models. This contrast does not occur at high doses where all three models are essentially the same and predict inhibitory/toxic effects, but rather at low doses where the hormetic model uniquely predicts a stimulatory and (often) salutary responses that are starkly different from the innocuous and toxic predictions of the prevailing threshold and LNT models, respectively. Therefore, compared to the threshold and LNT models, the hormetic model offers a unique stimulatory feature that not only is more sharply distinguishing, more readily identifiable and easier to study from a risk assessment perspective, but also may be explored further and possibly exploited for novel and mechanistically based advancements in the fields of medicine, biology and human performance.

The above enshrined argument to support the adoption of the LNT for cancer risk assessment by the 1972 BEAR Committee is based on assumed limitations of the threshold model and the evocation of a Precautionary Principle. This position became operational and was institutionalized in the US National Toxicology Program (NTP) when the decision was made for a chemical to be tested for cancer at the MTD and only a few very high fractional doses of the MTD. Since the number of doses is so limited and the doses so high, it became impossible to differentiate a linear from a threshold dose-response model as both models can fit the data for a vast majority of the studies. In effect, within a precautionary framework, a testing scheme was developed for cancer risk assessment by the government agencies that could never challenge the LNT belief. Despite this situation, a large series of scientifically based challenges to the credibility of the LNT for cancer risk assessment has been presented herein. These science-based challenges are substantial and cumulative and indicate that the LNT model simply has too many weaknesses for its credible use in public policy. Perhaps this should not be too surprising as the LNT has never been based principally upon science but rather upon a protectionist philosophy [47] (BEAR-Genetics and Medical Panels, 1960) (see Calabrese [48]) that was poorly constructed and likely wrong as well. Despite a large body of scientific concerns surrounding the theory and use of LNT, it appears to make little, if any, difference in the regulatory world. EPA administrators will claim that their science is transparent and sound, but in the case of their prime risk-assessment tool, the LNT, this is anything but the case. With respect to the BEIR I [65] Report the claim was that the threshold alternative to the LNT was not viable because of a "lack of sufficient theory of radiation (and chemical) carcinogenesis from which the concept (of "Threshold Dose") may be deduced and an empirical demonstration has not been made." It is obvious that LNT is no longer the answer, but what might be its alternative?

17. The BEIR I (1972) error: how it institutionalized the LNT myth

As noted in section 12, the BEIR I [65] committee "institutionalized" the LNT model for human cancer risk assessment, giving it more extrapolative credibility by switching from Muller's fruit fly to the Russells' mouse model. It did so while avoiding the use of the Russells' female mice data that demonstrated a striking threshold effect (at 27,000-fold greater than background) and defaulting to the male mouse data that suggested (but did not quite achieve) a threshold, thereby preserving the LNT mantra of the BEAR I Genetics Panel. The Committee's LNT recommendation was then adopted directly by EPA in 1975 and has continued in use to the present, becoming almost an enshrined regulatory belief by society. However, the Russells' nearly two million mega-mouse database would "quietly" but seriously be challenged some 25 years later by Paul Selby, a geneticist at ORNL, who worked with the Russells' for his entire career. Selby discovered important irregularities in the historical controls that had been used in all risk assessment/dose response analyses, including the judgments of BEIR I [65]. Selby brought these irregularities to the attention of high-level officials at the U.S. Department of Energy. A formal external evaluation ensued and concluded that serious errors had occurred in the controls that needed to be corrected. The external Panel recommended that the Russells' [70] and Selby [67,68] independently publish their corrections in the peer-review literature. The Russells' report acknowledged the need for correction and increased the background mortality rate upwards by 120%, while Selby's correction factor was even greater. This striking development received little notice in the literature due to its highly technical nature and the strong general inclination to avoid personal attacks. However, after about 20 years I became aware of the Russell-Selby debate and obtained much of the detailed background material, the external panel write-ups, and prolonged telephone interviews with Selby. In the final analysis, the Russell correction [70] of the male mouse (spermatogenic) data directly challenged the LNT model and yielded a threshold response for the males and a hormetic response for the females [227,228]. Had the Russell correction been available to the BEIR I [65] Committee, the LNT model may not have been recommended or adopted by the EPA since the corrected data directly refuted the scientific basis upon which the Committee had issued its LNT recommendation. Thus, the LNT recommendation of the BEIR I [65] Committee was based on a clear and profound mistake that was subsequently adopted by the EPA in 1975 [73] and continues to be applied uncorrected to this day in the estimation of cancer risks despite new knowledge and an awareness of past errors.

18. Model uncertainty

Trying to resolve deep-seated disputes over what model should be used by regulatory agencies for cancer risk assessment is problematic due to ideological issues, scientific uncertainty, and lack of capacity for adequate model validation. Given this current state of affairs, Calabrese et al. [229] have proposed a scientific compromise that involves the integrative participation of the three most substantial models available today – the threshold, LNT, and hormesis. They proposed that the LNT should serve as the upper bound and the hormetic as the lower bound of risk, with the threshold model providing the intermediate position. Of importance is that the nadir of the hormetic response coincides with the safe dose of the threshold model using 100-fold uncertainty factor (UF) when based on lifetime animal studies. This dose also corresponds to the 10^{-4} risk estimate of the LNT model. Such an approach is scientifically superior to the 10^{-6} risk estimates currently in use since the LNT risk estimates at 10^{-4} would have less uncertainty and also converge on the risk estimates of the other two models. Considerable public health benefits and cost savings would be experienced if the science in support of hormesis and hormesis itself were to be recognized and adopted. However, under the current LNT guidance, the notion of a clearly

defined science-based risk would remain illusory and no extra benefits or cost savings could be expected to accrue to society. Combining essential components of all three models into a less uncertain hybrid model is proposed as a workable compromise to yield a practical, science-based alternative to LNT [230].

19. LNT and evolution

It has long been recognized that the occurrence of cancer is a function of age, increasing in frequency to the 6th or 7th power of age. Thus, age becomes the most dominant risk factor for most cancers. This observation is consistent with findings that endogenous metabolism affects vast numbers (i.e. in the millions) of genetic damage events occurring in each cell each day. Highly efficient constitutive repair processes reduce this total to an average of about one mutation per cell each day, an efficiency which is greater than 99.9999%. Despite such an impressive repair efficiency, cells will progressively accumulate genetic damage, averaging about 400 unrepaired mutations per cell per year. By the time one reaches 80 years of age, each cell is estimated to have accumulated some 30,000 nuclear mutations. This perspective indicates that cells are not passive, but very dynamically involved in the damage and repair process. Furthermore, it is also well documented that external radiation can upregulate adaptive responses via the process of hormesis and further enhance repair processes by approximately 30–60% when exposures are increased up to about 100 fold times greater than background. Such upregulated hormetically-based adaptive repair processes usually stay active from one to several weeks and can be reactivated. During this period the upregulated responses not only significantly reduce background genetic damage but also establish a type of biological resilience that confers substantial protection against highly toxic exposures to the same or similar agent within a type of preconditioning framework. This capacity to adapt to endogenous and exogenous damaging exposures with a highly efficient detection and repair process clearly indicates that cells can respond with striking reparative efficiencies. These are the characteristics of cells that have evolved the means to survive within highly hostile environments. Thus, these cells are not passive entities to induced damage caused by the external environment.

The LNT dose response is one in which all damage is assumed to be cumulative, non-repairable and irreversible. This is how it was originally articulated by the US NAS BEAR I Genetics Panel in 1956 when they made their recommendation that regulatory agencies switch from a threshold to an LNT dose response model. The BEAR Genetics Panel adopted both a scientifically and non-evolutionary framework, denying the possibility that biological systems were inherently defensive and would have multiple complementary and redundant adaptive systems that would prevent and repair cellular and genetic damage. Over the past 70 years, there has been the discovery of a plethora of adaptive mechanisms that can now account for how cells can repair millions of genetic damage effects per cell per day and also induce overcompensation repair processes via hormesis. These discoveries have shown that cells are not passive accumulators of damage like the BEAR Genetics Panel stated, but rather are dynamic living entities that detect, activate, repair, push back and actively engage their environment, including the massive numbers of damage events that they inflict each day to themselves. In fact, the LNT model may be considered an anti-evolution model. In contrast, the hormetic and threshold dose response models are built upon the concept of evolution, are framed within the context of damage detection, repair, modest overcompensation and recovery. It is the contention of the present paper that toxicological models that predict human disease need to be based on evolutionary principles, making the LNT model the least attractive model for realistic risk assessment.

20. Conclusions

The LNT model has many serious flaws that should preclude its continued use as the default model in cancer risk assessment. The model was born of an incorrect interpretation by Muller that he had produced single gene mutations (i.e., point mutation). This mistake inspired the creation of the LNT single-hit model several years later, which eventually was widely adopted and used by the US EPA and other national regulatory agencies (Table 1). However, this LNT single-hit model was founded on the flawed assumption that the observations of radiation-induced genetic damage was involved were really mutated genes and that gene mutation could not be repaired. Since the genetic damage was principally in the form of large chromosomal deletions rather than single mutated genes, this model and its risk assessment predictions were invalidated. The next flaw in the ontogeny of LNT was the decision to use mature spermatozoa without DNA repair to extrapolate cancer risk to other reproductive cells (e.g., spermatogonia, oocytes) and somatic cells with DNA repair. In the late 1950s, the NCRPM uncritically accepted these flaws and recommended the LNT single-hit model for use in cancer risk assessment, even though the NAS BEAR I Genetics Panel (1956) never addressed cancer risk assessment and the later BEAR Genetics and Medical/Pathology Panels (1960) (see Calabrese [34]) explicitly referred to LNT use for cancer risk assessment as having unacceptable uncertainties due to extrapolation from very high to low doses. In 1972, the US NAS BEIR I Genetics Subcommittee [62] ignored the recommendations of the 1960 BEAR Panels, reverting back to the 1956 flawed BEAR I Genetics Panel recommendations and unwittingly accepted results from the Russell mega-mouse study that contained

Table 1
LNT Chronology: From mutation to cancer risk assessment.

Statement	Year
Muller report on X-ray induced mutation in *Science*	1927
Oliver (Muller student) dissertation showing linear dose response for radiation induced mutations	1930
Muller proposes Proportionality Rule	1930
Timofeeff-Ressovsky et al. propose single-hit model without a gene repair feature and link to Muller's linear dose response mutational data	1935
Ray-Chaudhuri (Muller's student) dissertation supports total dose/linear theory	1939
Manhattan Project-genetic mutation study starts at U. Rochester with Curt Stern directing project	1943
Ernst Caspari's data support threshold rather than linear dose response	1946
Stern published Warren Spencer and Caspari papers in Genetics	1948
Stern and Uphoff publish mini-meta analysis of Manhattan Project mutation research in *Science*	1949
National Academy of Sciences BEAR I Genetics Panel	1955–1956 recommend switch to LNT, 1956
NCRP applies LNT model for cancer risk assessment	1958
William L. Russell (Oak Ridge National Labs) published first evidence of dose rate for mutations with ionizing radiation, suggesting the existence of DNA repair	1958
NAS BEAR II Genetics Panel, report acknowledges dose rate in mouse and Drosophila	1960
NAS creates BEIR I (1970) which retains LNT while rejecting total dose; it switches to use of Russell mouse data from fruit fly reliance.	1970–1972
EPA adopts LNT based on the use of the Russell data	1975
Paul B Selby reports error in Russell control group in 1995; error confirmed by the Russells and corrected in the scientific literature separately by Russells and Selby	1996 and 1998
Calabrese applies Russells' and Selby corrections to BEIR 1972 risk assessment and reports that a threshold or hormesis response would have been reported if the control group error had been detected and corrected at the time of BEIR I	2017

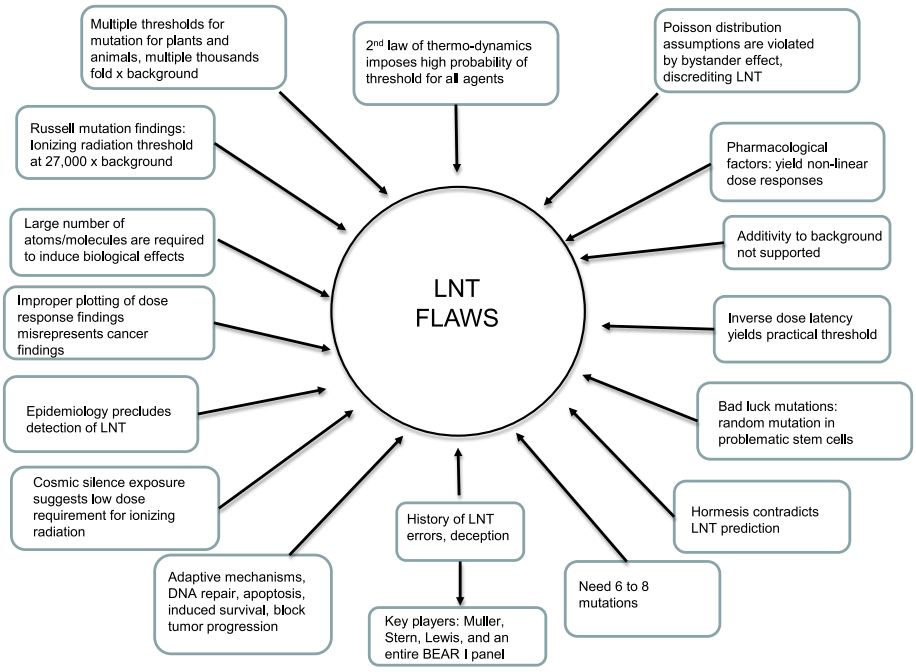

Fig. 6. LNT: Its multiple significant scientific limitations.

flawed control data and, as a result, wrongly recommended use of the LNT [225,226], which was later adopted by the fledging US EPA [70] as the basis for their cancer risk assessment for ionizing radiation and chemical carcinogens.

This recapitulation of the LNT history describes a process that was in many ways was strategically formulated and directed by leaders in the radiation genetics community, linked to powerful interests of the US NAS and the Rockefeller Foundation to orchestrate a profound dose response revolution. As has been reported elsewhere in detail [24,48,51], this was achieved via a series of errors, obfuscations, deceptions, scientific misconduct at the highest levels, the blatant use of political power to stack committees to achieve policy goals, the enticement of academic NAS BEAR Genetics Panel members with funding promises by Weaver,[15] the manipulation of influential media outlets, and an arrogant refusal by the NAS BEAR Genetics Panel to provide the public with a scientific rationale for recommendations. Any serious recounting and digesting of this dose-response/risk-assessment history shows that the basis of the cancer risk assessment in the US and worldwide represents a major failing and embarrassment to the radiation genetics community, the US NAS, NCRPM, the US government, especially the EPA and the world-wide scientific community.

In addition to its flawed history, LNT displays multiple significant limitations that preclude its use as a default model by regulatory agencies and toxic tort risk-assessment activities (Fig. 6). These include:

1. LNT has not been validated in experimental and epidemiological studies despite massive efforts to do so.
2. Large numbers of studies in the peer-reviewed literature directly contradict the LNT model in the toxicological and epidemiological literature.
3. Many complementary and redundant adaptive responses operate at the cell, organ and individual levels that intervene to block, repair and/or reverse the initiation, promotion and progression of carcinogenesis.
4. The major factor in approximately 70% of human cancers involves random mutations occurring in actively replicating stem cell populations. This observation associates a high proportion of human cancers largely to Bad Luck, i.e., random mutations to key stem cells. Although other aspects of the carcinogenesis process can affect the remaining course of the disease, the pure randomness of human cancers at the level of the individual and the stem cell is an important element that markedly weakens the LNT concept.
5. All eight principles of the US NAS that guided the adoption of the LNT in the late 1970s and early 1980s have been shown to be invalid and/or not verifiable [191]. The LNT model was adopted by government agencies based on numerous fears, misunderstandings, important scientific misrepresentations, and errors, all within the context of a highly precautionary perspective. Over the past 50 years, the historical and scientific bases for these actions have been shown to be essentially incorrect and have improperly guided the process of cancer risk assessment, profoundly affecting society, public health, medical treatments, and vast numbers of policy and legal decisions.
6. Given the serious flaws of the LNT model and the contentiously irresolvable nature of the cancer risk-assessment debate at low doses, a model optimization compromise approach to reduce uncertainty is recommended that integrates the optimized features of the LNT, threshold, and hormetic dose-response models.

[15] On pages 35 of the BEAR I Genetics Panel transcripts of Feb. 5, 1956, Chair Weaver stated: "There may be some very practical results—and here is the dangerous remark—don't misunderstand me. We are just all conspirators here together. I am not talking as an officer of the Rockefeller Foundation, but I will bat my head in the Rockefeller Foundation to try to get a very substantial amount of free support for genetics if at the end of this thing we have real case for it. I am not talking about a few thousand dollars, gentlemen. I am talking about a substantial amount of flexible and free support of genetics. I will bat my head off to get it … …"

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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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