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An examination of the linear no-threshold hypothesis of cancer risk assessment: Introduction to a series of reviews documenting the lack of biological plausibility of LNT

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The linear no-threshold (LNT) single-hit dose response model for mutagenicity and carcinogenicity has dominated the field of regulatory risk assessment of carcinogenic agents since 1956 for radiation [8] and 1977 for chemicals [11]. The fundamental biological assumptions upon which the LNT model relied at its early adoption at best reflected a primitive understanding of key biological processes controlling mutation and development of cancer. However, breakthrough advancements contributed by modern molecular biology over the last several decades have provided experimental tools and evidence challenging the LNT model for use in risk assessment of radiation or chemicals. Those science advancements have revealed that DNA is not simply an inert chemical target such that even a single "hit" potentially results in cancer, or that multiple hits additively cumulate over time. Modern biology has now unequivocally demonstrated that biological systems mount a plethora of highly integrated defenses to a continuous chorus of endogenous and exogenous attacks (e.g., ROS) on core genetic material and function. These defenses (expressed at subcellular, cellular, organ and whole body levels) are essential to sustaining cell and organism homeostasis. This massive explosion in fundamental understanding of cell and organism function now clearly points to the need to examine the impact of this vast body of knowledge on the scientific legitimacy of maintaining the LNT model as a continuing and scientifically defensible driver of radiation and chemical carcinogen risk assessment.

The concept of LNT responses to exogenous agent exposures had its origins well before its application in carcinogen risk assessment when it was first proposed as the mechanistic explanation of biological evolution. Inspired by the research of Hermann J. Muller demonstrating that very high doses of radiation induced transgenerational phenotypic changes claimed as caused by heritable point mutations [18], two physical chemists from the University of California at Berkeley proposed that cosmic and terrestrial ionizing radiation provided the mechanistic driving force for the evolution of life on earth [22]. Despite this perceived need to identify a plausible mechanistic explanation for evolution and the prominence of co-author Gilbert Lewis, who would be nominated for the Nobel Prize some 42 times, this idea generated much heat but little light. This hypothesis was soon found to be unable to account for spontaneous mutation rates, underestimating such events by a factor of greater than 1000-fold [19].

Despite this rather inauspicious start for the LNT model, Muller would rescue it from obscurity, giving it vast public health and medical implications, even proclaiming it a scientific principle by calling it the Proportionality Rule [20]. While initially conceived as a driving force for evolution, Muller gave the LNT concept a second chance at scientific life and tirelessly promoted it as a plausible basis for radiation safety assessment for the remainder of his scientific career. Muller soon would link a mechanism to his model via the collaboration of leading physicists who saw creative advances occurring at disciplinary interfaces, such as genetics and nuclear physics. Soon this interdisciplinary grouping would devise a mechanism via target theory for Muller's data and the LNT-single hit model was born [6,24].

Technology in the form of X-and gamma ray generation and their associated medical applications and the ensuing development of nuclear weapons would provide the impetus for propelling scientific recognition and application of LNT to societal risk concerns. This early knowledge of mutation and its proposed mechanism and associated LNT-driven dose response features would become transformed into a massive public policy issue following the dropping of the two atomic bombs in 1945. Society became terrified of the thought of generations of deformed children and predictions of a plethora of inescapable cancers arising from the rapid proliferation of these new ionizing radiation based technologies [3,4,7].

As for Muller, his now decades old research discovery took center stage and he was soon awarded the Nobel Prize in December of 1946 for his discovery of radiation-induced gene mutations in fruit flies. Notably, while unequivocally stating in his Nobel lecture that "*there is no escape from the conclusion that there is no threshold for radiation-induced mutation*", as a paid consultant, Muller had seen the results of a classified

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study from the Manhattan Project by Ernst Caspari which demonstrated a clear mutation threshold when a lower radiation dose rate was used [5,12]. As a relevant aside, Caspari's threshold findings have been replicated in more recent fruit fly studies with additional observations of low-dose hormesis [16,21]. Following his Nobel Prize, Muller now had the public and scientific platform he had so long sought and he capitalized on this, leading radiation geneticists on a quest to exchange longstanding regulatory beliefs in a threshold dose response model for mutagens and carcinogens for the LNT-single hit model. This objective required some critical orchestrated political and financial assistance at a key strategic time from the Rockefeller Foundation and the US NAS (both organizations ironically headed by Detlev Bronk), but Muller and his radiation geneticist colleagues ultimately prevailed in bringing about a profound change in risk assessment policy in 1956, convincing members of the Genetics Panel at the US NAS to recommend the adoption of LNT for low-dose radiation cancer risk assessment [7,9].

The fear of radiation-caused cancer was soon extended to chemicals by the US congressional passage of the “Delaney clause” in the Food Additives Amendment of 1958, which pronounced that any new substance found to cause cancer in humans or animals, regardless of dose, could not be used as a food additive. Soon thereafter in 1959 the Secretary of the US Department of Health, Education and Welfare created the first wave of chemical cancer panic by declaring low-level residues of the herbicide aminotriazole in cranberries to be a cancer threat. This pesticide had recently been reported to induce cancer in experimental animals at very much higher test doses (equivalent to humans eating 15,000 pounds of cranberries every day for many years). That warning, which occurred during the week of Thanksgiving, caused an immediate plummet in the sales of this holiday staple and was a key catalyst for decades of ensuing public cancer chemophobia [11,17].

These early profound shifts in the basic approach to cancer risk assessment, all of which were based on mostly poorly-understood toxicological phenomena of the time, had major ripple effects. Perhaps most substantively, they provided key inspiration to Rachael Carson in her seminal [13] book, *Silent Spring*. Her book awakened and scared both the world and President John F. Kennedy with concerns of widespread chemical contamination, especially in the form of chlorinated pesticides such as DDT. It was indeed her influence that would further fuse and synergize with Muller's LNT revolution, leading the US Congress to create the National Environmental Protection Act (NEPA) in December of 1969 and Environmental Protection Agency (EPA) only months later. While Muller would die in 1967, his transformational advocacy of LNT would find their target and inspire the actions of the soon-be-created US regulatory agencies.

In 1972, a new NAS Committee i.e., Biological Effects of Ionizing Radiation (BEIR I), would “revalidate” the LNT using a mammalian rather than fruit fly model based on the massive research by William Russell at Oakridge involving more than two million mice. The LNT concept was preserved despite the recognition that the BEAR I had made a crucial error in claiming that risk was based on total dose rather than dose rate. The re-affirmation of LNT by BEIR I, based on the Russell work, was adopted by EPA as the “gold” standard in 1975 for radiation and chemical carcinogens. It provides the key fundamental grounding and rationalization for the LNT. This was crucial since epidemiological methods were incapable of quantitatively assessing the shape of the dose response in the low dose zone due to background variation and other methodological limitations. The Russell studies and the BEIR recommendation would become a toxicological/risk assessment “homing” approach that would help to ensure the adoption and continued use of LNT. This scientific foundation for LNT has been challenged since, as it has been learned [10], the Russell control group mutation estimates were substantially flawed. After appropriate corrections, the massive data revealed not only a clear mutation threshold in male mice, but an hormetic response in female mice. These unequivocal findings, which have never played a meaningful role in EPA's subsequent regulatory policy, further erode trust in use of LNT in science-supported cancer risk assessment actions.

By the mid-1970s the Carson-inspired environmental revolution was fully engaged, with the emerging belief that 80% of human cancers were due to environmental factors, mostly manmade, and therefore giving hope of potentially eliminating this dreaded disease with strong regulations. Driven by such beliefs as well as the obvious visual recognition at the time that air and water pollution needed to be comprehensively addressed, Congress was inspired to create a plethora of environmental legislation and an associated aggressive and well-funded research framework “to end cancer in our lifetime”.

The emerging regulatory efforts were more successful than Muller and Carson could ever have imagined. By the mid-1970s the LNT model would be adopted by EPA for risk assessment of both ionizing radiation and chemical carcinogens, with no dose, even a single ionization or molecule, absent of potential harm. Before society really knew what happened, there was an environmental transformation, actually an intellectual and emotional revolution, that could be seen in many ways, such as trying to manage industrial waste practices, toxicity screening of new chemicals prior to entering commerce and those already in wide use, drastically reducing automobile emissions, profoundly reducing lead in the environment, and many other such actions. These and other actions also created profound fears of “toxic” environmental and occupational agents even at extremely low doses. These activities were soon seen and felt in the cost of new environmental and occupational regulations, massive clean-up costs for all types of contamination, leaving many in the regulated community to wonder “how clean is clean? What is clear is that LNT-based cancer risk assessments resulted in estimates of acceptable environmental exposures and implementation of regulatory actions that were increasingly challenging, if not impossible, to meet, and with every additional increment of exposure reduction often resulting in disproportionate clean-up and/or control standards. For example, with respect to remediation of radiation-contaminated sites, Pete Lyons, then with the Department of Energy (DOE), noted that removing soil down to 25 mrem instead of 15 mrem would save billions of dollars in unnecessary remediation costs (personal communications with Robert Golden).

Nonetheless, the revolution had occurred on multiple levels and it happened so fast that it essentially outstripped needed scientific foundations upon which to base regulatory decisions. In 1976 the EPA created the Cancer Assessment Group (CAG) that was quickly followed by an interagency report lead by David Hoel (NIEHS) and David Gaylor (FDA), two leading biostatisticians, which recommended the LNT-single hit model. The CAG, under political pressure to establish functional cancer risk assessment practices, reached into the NAS BEAR and BEIR radiation risk guidance documents to quickly adopt the LNT-single hit model and apply it for chemicals and radiation based on the assumption that both types of carcinogens acted via a mutational mechanism that was assumed as linear at low dose. At the same time Kenny Crump, David Hoel and others co-authored a paper proposing that chemically induced tumors acted in an additive to background manner, explicitly following identical no-threshold mechanisms [14]. The additive to background assumption was subtle but significant. Its acceptance would essentially assure that linearity would occur under virtually all modeled situations, and surprisingly, even when the dose response data reflected a threshold. It took a while for this assumption to guide EPA cancer risk assessment practices, but it has done so now for more than 30 years, with no challenge and little reflection, becoming an irrefutable fixture to the risk assessment process [11].

The environmental revolution was a *fait accompli* by the early 1980s, with the OSHA Carcinogen Hearings from 1978 to 1980 assuring the final codification of LNT-based risk assessment practices. These activities were also supported by complementary efforts of the NAS Safe Drinking Water Committee (SDWC) created in 1975 in response to passage of the US Safe Drinking Water Act in 1974. The SDWC deliberations were powerfully influenced by key players such as David Hoel and the director at the National Institute of Environmental Health

Sciences (NIEHS), David Rall, in addition to two members of the original 1956 BEAR committee. Their strong advocacy for the LNT model set up a virtual storm of controversy amongst the toxicologists who argued that the threshold/safety factor approach was more than adequate to ensure protection from potentially carcinogenic chemicals. Ironically, and not generally appreciated, is that the next SDWC, under the leadership of toxicologist John Doull (to whom this Special Issue is dedicated), rescinded the NAS endorsement of LNT with the suggestion that data determine the dose-response model. However, the next SDWC reluctantly re-endorsed LNT which has remained in place to the present.

Functional consolidation of LNT was now essentially complete and in 1979 EPA would promulgate its first LNT-based drinking water standard for trihalomethanes (THM) affecting numerous public drinking water supplies using chlorinated disinfection processes. The costs to society were enormous, forcing many large cities to spend huge amounts of money on new or refined disinfection technologies. This was just the start of similar patterns affecting many aspects of society that were increasingly dependent on radiation- and chemical-based technologies to support modern and higher quality lifestyles. LNT was now the “law of the land”, and additivity to background (the validity of which has been significantly challenged [11]) would assure it could never lose. For all practical purposes the LNT had assumed the scientifically inappropriate position of the null hypothesis, which scientific convention would otherwise require starting with the assumption that very low doses to radiation and chemicals do not cause mutation or cancer [23]. Because the LNT hypothesis assumes that even a single ionization or molecule “hit” is capable of inducing mutation and ultimately cancer, the new “null” hypothesis essentially became *de facto* non-falsifiable.

Public fears that society was swimming in a sea of man-made carcinogens were further amplified by the 1973 technological advancement by Bruce Ames that used inexpensive and easily-conducted bacteria-based experimental systems to detect chemical mutagens. Relatively suddenly, large numbers of anthropogenic environmental chemicals were flagged as mutagens, and under the banner of existing LNT dogma, were considered as potential human carcinogens. These fears were further compounded by reports of approximately 50% positive cancer responses in animal bioassays using Maximum Tolerated Doses (MTD), even though such doses often were very much higher than real-world human exposures [15]. These whole animal findings, coupled to the in vitro mechanistic evidence of genotoxicity in the “Ames” assay, virtually assured defaulting to LNT dose-response risk assessments. This latter point was important for it ensured that the toxicological and risk assessment rules were “set”, and outcomes were assured in any battle over threshold versus LNT for particular chemicals. Ironically, Ames later realized that many thousands of naturally occurring chemicals, including many present in clearly “healthy” diets of fruits and vegetables, also were mutagens in genotoxicity testing systems. And even more importantly, he recognized that human exposures to these natural substances *in toto* and in some cases individually were far greater than exposures to anthropogenic agents. This fundamental observation led Ames to vehemently challenge the assumption that exposures to man-made chemicals were, with few exceptions, primary contributors to human mutagenicity and cancer [1].

In the aftermath of this now nearly half-century environmental revolution, what has been accomplished and learned? Great progress has been made in the cleaning and greening of the environment. The air and water in developed countries are indisputably far cleaner today compared to the 1960s. All of this is good news, and was a highly worthwhile focus of attention. However, after many trillions of dollars spent worldwide in efforts to clean up the environment to avoid promised cancer epidemics, with the exception of smoking (which directly delivers high doses of potent carcinogens to the lungs), there has been essentially little impact on the overall incidence of age-adjusted cancer in the US and in the rate of deaths from cancers per year [2]. Observed “increases” in certain cancers (e.g., breast, prostate, thyroid) can in part be attributed to improved surveillance and/or diagnosis which are not environmentally driven. Early post-WWII generations, despite indisputably experiencing the highest levels of environmental pollution during the 1950's to 70's, are not exhibiting the cancer epidemic otherwise expected from LNT assumptions. The cancer statistics are strikingly, surprisingly and depressingly essentially static. This suggests that despite commitment of enormous societal resources to comply with LNT-based risk assessments, LNT-based cancer regulatory practices have failed to fulfill the promise of making meaningful differences in overall cancer incidence and mortality.

Would society have been better served by the threshold model? Certainly, advances in modern biology as described in some of the papers of this Special Issue exposed the lack of biological plausibility of the LNT. And as observed by Ames, it should be deeply troubling to all health professionals that the LNT paradigm counterintuitively infers that the greatest risks for environmentally-induced cancer are due to consumption of foods, i.e., fruits and vegetables, that otherwise are viewed as the baseline for the healthiest of human exposures.

The problem then is whether rapid and profound advancements in biological sciences are sufficient and appropriate to displace a default LNT-based risk paradigm that was undeniably structured on simplistic and erroneous interpretations of 1940's era biological experimentation. It is clear that in no other scientific endeavors, other than LNT-based cancer risk assessments, has the practical progression and application of advancements in science knowledge been so irrevocably held hostage to what not only is a functionally untestable hypothesis but also what is now robustly counter to modern biological science.

It was in the above-noted spirit of thinking and concern that this project convened a group of highly qualified experts in toxicology, radiation biology, epidemiology and related areas to undertake a systematic review of the scientific validity of the LNT model from both historical as well as modern molecular biology perspectives. This approach entailed a fresh look at Muller and his historical contributions and brings it up to the present with modern molecular biology-driven considerations of mutation and cancer mechanisms and how they inform the dose-response of environmental radiation- and chemically-induced human cancer. These perspectives have been integrated into a comprehensive evaluation of the LNT model, examining whether this risk assessment strategy that has dominated the past half century is scientifically sustainable.

Given the enormous and increasingly precious societal resources required to address the broad functional implications of the LNT risk assessment paradigm, the stakes are too high for the scientific and regulatory communities to continue to ignore the ever-growing mountain of evidence directly challenging the biological underpinnings of the LNT model. The primary objective of the perspectives presented in the series of papers comprising this special issue of Chemico-Biological Interactions is to catalyze a serious reexamination of likely the most influential and costly risk assessment practice that should have been recognized as substantially flawed from its inception. Such a need is further emphasized by the fact that, book-ending Muller's 1946 Nobel, the 2015 Nobel Prize for chemistry was awarded to three scientists from the Francis Crick Institute, Duke University and the University of North Carolina for their detailed mechanistic studies of three different kinds of DNA repair (i.e., base excision repair, mismatch repair and nucleotide excision). It is these and countless other complementary cellular mechanisms which describe the evolutionarily-conserved and multilayered processes that function for the important purpose of protecting DNA integrity, and unquestionably are modulators of mutational and cancer thresholds associated with low dose radiation or chemical exposures.

It was not an objective of this project, however, to propose alternative approaches to cancer risk assessment beyond LNT, other than to build the case that both history and present-day biological knowledge justify moving from LNT to threshold-based risk assessments. It is clear

that continuing advances in toxicological and biological sciences are revealing a multiplicity of threshold-based options that will support substantially improved science-versus policy-based risk assessments.

It is the hope of the contributing authors of this Special Issue that this volume (Calabrese, E., 2019; A comprehensive assessment of its historical and scientific foundations; Costantini, D. and Borremans, B. The linear no-threshold model is less realistic than threshold or hormesis-based models: An evolutionary perspective; Scott, B. The LNT model for cancer induction is not supported by radiobiological data; Tharmalingam et al.; Re-evaluation of the linear no-threshold (LNT) model using new paradigms and modern molecular studies; Brooks, A. The impact of dose rate on the linear no threshold hypothesis; Andrew, M. Zarnke et al. BEIR VI radon: The rest of the story; Kobets, T. and Williams, G. Review of the evidence for thresholds for DNA-reactive and epigenetic experimental chemical carcinogens; Clewell et al. Mechanistic aspects of chemical carcinogens demonstrating thresholds; Ricci, P. and Tharmalingam, S. Ionizing radiation epidemiology does not support the LNT model; Williams, R.A. Economic benefit-cost implications of the LNT model.) will stimulate a broad scientific and policy reevaluation of cancer risk assessment policy and practices. The flawed history of LNT and its lack of biological plausibility as revealed by robust advancements of modern-day biological sciences should compel regulatory agencies to immediately remove it as the default model in cancer risk assessment. Indeed, the time is ripe, if not long overdue, to seize the opportunity to place cancer risk assessment on sounder, more biologically based and fully transparent foundations.

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Appendix A. Supplementary data

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Transparency document

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