Environmental Research 166 (2018) 175–204

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Review article

The additive to background assumption in cancer risk assessment: A reappraisal

Edward J. Calabrese

Department of Environmental Health Sciences, University of Massachusetts, Morrill I, N344, Amherst, MA 01003, United States

ARTICLE INFO

Keywords:
Cancer risk assessment
Linear non-threshold dose-response
Oncogenes
Threshold dose-response
Carcinogen mechanism

ABSTRACT

The assumption that chemical and radiation induced cancers act in a manner that is additive to background was proposed in the mid-1970s. It was adopted by the U.S. Environmental Protection Agency (EPA) in 1986 and then subsequently by other regulatory agencies worldwide for cancer risk assessment. It ensured that cancer risks at low doses act in a linear fashion. The additive to background process assumes that the mechanism(s) resulting in induced (i.e., treatment related) and spontaneous (i.e., control group) cancers are identical. This assumption could not be properly evaluated due to inadequate mechanistic data when it was proposed in the 1970s. Using the findings of modern molecular toxicology, including oncogene activation/mutation, gene regulation, and molecular pathway analyses, the additive to background assumption was evaluated in the present paper. Based on published studies with 45 carcinogens over 13 diverse mammalian models and for a broad range of tumor types compelling evidence indicates that carcinogen-induced tumors are mediated in general via mechanisms that are not identical to those affecting the occurrence of the same type of spontaneous tumors in appropriate control groups. These findings, which challenge a fundamental assumption of the additive to background concept, have significant implications for cancer risk assessment policy, regulatory agency practices, as well as fundamental concepts of cancer biology.

1. Introduction

This paper assesses a critical, but overlooked area of cancer risk assessment (i.e., cancer dose-response assessment), the additive to background assumption, that essentially ensures low dose linearity in the estimates of carcinogen exposure risks. This assumption was proposed for application to cancer dose-response assessment by Crump et al. (1976). A decade later it was incorporated into governmental risk assessment policy and practices during 1986 (Anderson et al., 1983; Crump, 1984; U.S. EPA, 1986) and has continued to the present (U.S. EPA, 2005; EFSA, 2017). This assumption was proposed during the mid 1970s when it was not possible to assess its scientific validity with the oncogene revolution starting in the mid-1980s and the continued clarification of molecular mechanisms for spontaneous and induced tumors to the present. It is now possible to evaluate the scientific validity of the additive to background assumption. The present paper demonstrates that the additive to background assumption that spontaneous and induced tumors occur via identical mechanisms is not compatible with the vast body of modern molecular findings. Prior to assessing the additive to background hypothesis, a brief historical reconstruction of how linearity at low dose was adopted for cancer dose-response assessment by U.S. regulatory agencies during the 1970s is presented, providing the necessary scientific and regulatory contexts and introduction needed to assess the additive to background assumption.

2. Historical foundations of cancer risk assessment

2.1. The Thanksgiving Cranberry Scare of 1959

Within five years following the National Academy of Sciences (NAS) Biological Effects of Atomic Radiation (BEAR) I Genetics Panel report (NAS/NRC, 1956) recommending the use of linear dose response modeling in risk assessment, Nathan Mantel and Raymond Bryan (1961) would publish their landmark paper on cancer risk assessment. The modestly entitled paper, *Safety Testing of Carcinogenic Agents,* was based on the use of the tolerance distribution probit dose response model. The probit model was originally derived to assess non-carcinogenic responses (Zeise et al., 1987). However, Mantel and Bryan (1961) generalized its use, applying it to modeling responses of carcinogens. Their efforts followed by nearly two decades the earlier work of Bryan and Shimkin (1943) who applied the probit model to estimate cancer risks for several carcinogenic hydrocarbons based on chronic studies with male C3H mice, a study that suggested an hormetic dose response that was not addressed by the investigators.

E-mail address: edwardc@schoolph.umass.edu.

https://doi.org/10.1016/j.envres.2018.05.015
Received 9 April 2018; Received in revised form 8 May 2018; Accepted 11 May 2018
Available online 08 June 2018
0013-9351/

What stimulated the reemergence of interest in quantitative estimates of cancer risks was U.S. presidential politics. Mantel was employed as a biostatistician for the U.S. National Cancer Institute (NCI) during the time of the 1960 presidential election, pitting John F. Kennedy against Richard M. Nixon. During the run up to the election, there was the so-called Thanksgiving Cranberry Scare of 1959. The event proved to be both a major chemical scare for the American public and a chance for the two presidential candidates to demonstrate that they were not afraid of a small dose of the cancer causing (i.e. thyroid cancer) herbicide in their cranberry sauce or juice (i.e. Nixon had four servings of cranberry sauce while Kennedy had two drinks of cranberry juice-of course on the same day.) (http://coldwarstudies.com/2017/11/15/the-cranberry-scare-of-1959). The agent, 3-amino-1,2,4-triazole, which had been approved in 1957 for use on Cranberry bogs only after harvest, had been found in several sources of cranberries in the weeks leading up the Thanksgiving holiday (note that the farmers did not follow the instructions properly; they were only supposed to apply the herbicide after harvesting but applied it before). It became a political story when the Secretary of Health, Education & Welfare (HEW), Arthur Sherman Flemming went public on November 9, 1959 with the recommendation to the public not to buy cranberry products that year. His actions resulted in what might be called a consumer panic, which then threatened the livelihood of the cranberry industry. In an effort to prevent a similar public backlash in the future, Secretary Flemming asked the NCI for guidance on which cancer causing agents could be considered "safe" and what may be a safe or acceptable dose. To the rescue would come Mantel and the laboratory animal model cancer researcher Bryan, who were asked by the Director of the NCI to provide the needed guidance, including issues such as how to design appropriate animal bioassays and how to estimate risks and establish a means to distinguish between safe and unsafe. Little did the Secretary of HEW and the NCI Director realize that they had just opened a scientific version of Pandora's Box, with issues that still confront politicians, scientists and the general public.

In their publication, Mantel and Bryan (1961) would emphasize the generality of their dose response model approach for other agents and tumor endpoints. They introduced the concepts of no threshold and acceptable risk within a public health policy framework. In a manner to illustrate its practical utility they expressed the outcome of their model estimate in public health terms suggesting an acceptable risk with a value sufficiently low that few would have concerns over, that is, one cancer per 100 million people per lifetime. While this effort in 1961 by Mantel and Bryan was thought to have put a lid on concerns with chemical carcinogens, it was only the beginning, as Rachael Carson would publish her *Silent Spring* book a year later (Carson, 1962). The Carson publication, which was partially inspired by the efforts of radiation geneticist Hermann J. Muller, would galvanize the fledging environmental movement, lead to the creation of the National Environmental Protection Act (NEPA) (1969) and the EPA (1970) and help spark efforts to address the issue of cancer dose-response assessment about a decade later.

2.2. U.S. EPA, Cancer risk assessment, and low dose linearity

It would take about 12 years but the U.S. FDA would eventually restart its cancer risk assessment agenda by formally proposing the Mantel-Bryan (1961) model while still retaining the 1/100 million acceptable risk level in their July 19, 1973 (U.S. FDA, 1973) cancer risk assessment announcement in the *Federal Register*. As the regulatory stakes had changed since the Cranberry scare of 1959, this proposal was taken seriously, and became stalled in the U.S. regulatory apparatus. It finally emerged following what could only be seen as a rather elephantine-like gestational period in 1977 (U.S. FDA, 1977), having survived a presidential election and new political leadership. The Mantel-Bryan probit model approach had been largely retained, although with a number of alterations, including the adoption of a new acceptable risk value of one in a million.[1]

The practical significance of such actions was that it became the risk estimate below which no further governmental regulatory actions would be initiated. This recommendation was placed within the framework of a public health safety response to carcinogen residues in food products. Even though it had taken a long time to get an approved cancer risk assessment process through the regulatory system, the FDA-approved Mantel-Bryan model became the first cancer dose-response assessment model officially adopted by a U.S. federal regulatory agency. The next change would not take so long. About two years later, the U.S. FDA (1979) would alter its approach by dropping the tolerance distribution Mantel-Bryan model approach, replacing it with a linear dose response model. The rationale for such a decision was due to the more conservative risk estimates of the linear model along with its conceptual simplicity and ease of risk calculation (Anonymous, 1979). In the low dose zone, the one hit model as initially proposed by Timofeef-Ressovsky et al. (1935) yields very similar risk estimates as a simplified linear model. Getting a federal agency to change its cancer dose-response assessment model only two years after a long incubation period should raise the proverbial "why"? In fact, the FDA's actions were the direct offshoot of the recommendations of a multi-governmental agency panel with FDA technical representation (biostatistician David Gaylor) that published their linear dose response recommendation (Hoel et al., 1975). It was simply a matter of being more conservative, simplifying the process and timing.

While the U.S. FDA was pursuing its cancer risk assessment methods and issues, so to was the U.S. EPA. The posturing and approaches that emerged from this fledgling environmental regulatory agency seemed somewhat confusing to the outside reader and the regulated community. Much of the initial conceptualizing on the issue of regulation of cancer causing agents emerged from the Rachael Carson-inspired need to address the issue of risks from pesticides. Thus, during major pesticide hearings EPA staff attorneys presented an intellectual blueprint of what amounted to a set of Agency "cancer principles". The new "Principles" reflected the Agency view that carcinogen exposures should not be permitted…..that is, prevented from occurring in the first place. While the goal of this Principle was to ban carcinogenic agents from the market place, it was quickly seen as simply unrealistic, though it could remain a goal (Albert, 1994; Calabrese, 2009, 2013).

What emerged from this process was EPA adopting a set of non-regulatory guidelines that could be applied to a generic cancer risk assessment process (U.S. EPA, 1976). This system would have considerable practical importance, as it would employ quantitative risk assessment on chemicals and engineering-based processes. This conceptual framework would be the functional lead-in for a critical paper by the EPA's Carcinogen Assessment Group (CAG) (Albert et al., 1977), which reaffirmed the LNT concept and justified it based on epidemiological studies for smoking and ionizing radiation and the dose response pattern of induced genetic mutations based on the Ames assay with bacterial strains lacking DNA repair. This paper by

[1] During this regulatory "incubation" period within the FDA, Mantel et al. (1975) would update the original (Mantel and Bryan, 1961) application of the probit model with an "improved Mantel-Bryan procedure". The original Mantel and Bryan (1961) procedure incorporated Abbott's (1925) correction to adjust for spontaneous tumor background. This new procedure would account for background/spontaneous tumors via the introduction of a new estimated parameter "C", the expected (spontaneous) incidence in untreated animals, with the subsequent application of Abbott's correction (Abbott, 1925; Zeise et al., 1987). It is likely that the adoption of the independent of background approach using Abbott's formula by Mantel et al. (1975) lead to EPA accepting this approach several years later when it was incorporated into the single-hit model (Costle, 1979) and later into the multi-stage model (Anderson, 1983). Mantel et al. (1975) noted the possibility of an alternative to the independent of background model, by proposing a scheme similar to the additive to background concept. In this scheme, the spontaneous tumor rate "represents the response to the load of the test agents and its equivalent ALREADY in the environment. The total load for an individual or animal is then the sum of its administered dose and its environmental load. This was similar to that proposed earlier by Albert and Altshuler (1973) and later by Crump et al. (1976), except that Crump et al. (1976) tied the background and induced tumors via an identical mutation mechanism as discussed later in the text.

the CAG (Albert et al., 1977), which offered no references to support LNT, provided the introduction for the key policy statement of the U.S. EPA Administrator, Russell E. Train (1977) to adopt the linear dose response for cancer risk assessment.

Two years later, on March 15, 1979, the new EPA administrator, Douglas Costle, reported in the *Federal Register* (U.S. EPA, 1979) that risk assessment for animal data is performed using the 'one-hit model' as given in the 1976 Interim EPA Guidance document (U.S. EPA, 1976). Costle would justify this decision on the basis that the one-hit model was endorsed by the four federal agencies of the Interagency Regulatory Liasion Group due to its very protective/conservative features, uncertainties in animal to human extrapolation, concerns over whether humans may be more susceptible than the animal model, the occurrence of human variability (i.e., interindividual variation) and "other unknown factors". However, use of the single-hit model was criticized in public comments of the proposed Water Quality Criteria for suspected carcinogens (U.S. EPA, 1979).[2] As a result, EPA changed from the single hit to the multi-stage model by November 1980 (Anderson, 1983).

In an historical assessment of this cancer risk assessment period, Albert (1994), chair of the CAG during the 1970s and early 1980s, indicated that the EPA accepted the LNT model that was being applied by the U.S. Atomic Energy Commission (AEC) to assess cancer risks from ionizing radiation data based on the Manhattan Project findings of Stern at the University of Rochester (Spencer and Stern, 1948; Uphoff and Stern, 1949; see Calabrese, 2015 for a review). Albert (1994) reaffirmed that the LNT model was a good fit for EPA since it had exquisite simplicity and therefore was readily understandable. This model also was attractive to the Agency since it had biological plausibility based on target theory as derived from a unique collaboration of leading physicists and radiation geneticists (Timofeef-Ressovsky et al., 1935). Of particular importance to the broad mission of EPA was that "any difference between chemical carcinogens and ionizing radiation could be waived aside as they both cause genetic damage…" The generalizing of the LNT for cancer risk assessment to include the domain of chemical carcinogens was a game changing decision that would significantly affect numerous societal and scientific issues.

This brief historical summary recapitulates how quantitative risk assessment evolved within the regulatory agencies from about 1960–1980. However, the initial quantitative modeling originated in the early 1950s and displayed divergent fundamental biological assumptions depending on the model. It is within the decade of the 1950s where the underlying biologically-driven quantitative models for both background/spontaneous and exogenously induced cancer incidence would provide the theoretical, mathematical and biological frameworks for the cadre of available approaches employed in regulatory agency based quantitative risk assessments that emerged in the mid to late 1970s. Despite the growing sophistication of quantitative modeling in the 1950s, these efforts received their intellectual and biological foundations from the seminal paper of Timofeef-Ressovsky et al. (1935) and follow-up papers (e.g. Zimmer, 1941) on the development of dose response modeling which was based on the capacity of X-rays to induce gene mutations in mature spermatozoa of *Drosophila* as originally reported by Muller (1927).

2.3. Cancer: Quantitative Risk Assessment and Biological Plausibility

The issue of quantitative risk assessment for carcinogens became a major consideration for regulatory agencies such as EPA in the mid-1970s. Such assessments were dominated with debates by biostatisticians concerning which biostatistical model to employ for risk assessment, with a significant priority being biological plausibility. The first efforts toward biological mechanism modeling were those of Iverson and Arley (1950) which were the direct offshoot of Timofeef-Ressovsky et al. (1935) and their subsequent papers. Their single stage model assumed that a key biological change or transformation occurs in a single target, typically the result of somatic gene mutation (i.e., somatic mutation theory – SMT). The model was built upon the use of a proportionality constant that represented the rate of cell transformation per unit of administered carcinogen, as well as the assumed carcinogen potency and the sensitivity/susceptibility of the target cells. The transformed cell increased cell number via an assumed monoclonal expansion that may become detected after a critical number of cell divisions.

While their model had serious biological limitations, it stimulated other efforts including those of Fisher and Halloman (1951) which transformed observations of age-dependent stomach cancer death incidence into a new biostatistical model for cancer risk assessment. The cancer incidence was estimated to occur with the 5th or 6th power of age. Fisher and Halloman (1951) assumed that this power law relationship could be mechanistically explained if there were 6 or 7 different cells that were altered/transformed/mutated (i.e., initiated) in a single tissue. These transformed cells would then become biologically integrated, yielding a developing tumor mass. This became known as the multi-cell theory (i.e., polyclonal model). This hypothesis developed a following in the biomedical community, being supported over several decades by prominent scientists as seen in the writings of Wright and Peto (1969), Jones and Grendon (1975) and others.

During the early 1950s, Muller (1951) and Nordling (1953) hypothesized that a single cell can transform into a tumor only if it experiences a number of mutational events (i.e., SMT application). From this theoretical basis would emerge the multistage model, with its quantitative foundations being developed soon thereafter by Stocks (1953) and Armitage and Doll (1954, 1957).

3. The additive to background assumption

3.1. Ensuring linearity at low dose

The origin of the additive to background cancer risk concept was first suggested by Platt (1955) in a letter-to-the-editor of the *Lancet*. He proposed that the principal effect of a carcinogenic agent is likely to alter the functioning of a cell in such a manner that its clonal expansive descendants proliferate at a greater rate than other nearby cells. He then suggested that if aging were seen as an extended process of numerous cell divisions then cancerous processes would be a function of such aging activities.

Based on this logic it was predicted that cancer would occur in the cellular descendants of the initially altered cells more quickly than in the neighboring "normal" tissue but only following a prolonged latent period. This initial suggestion of Platt (1955) was adopted,[3] formalized, and extended by Armitage and Doll (1957) into what became the additive to background hypothesis and the basis of the additive to

[2] Despite the apparent interagency convergence upon the LNT single-hit model, use of this model was criticized since it did not incorporate data from high doses. It derived the linear assessment via the use of a straight-line extrapolation employing the lowest statistically significant response. As a result of such dissatisfaction, the CAG modified its positon, switching from a single-hit to a multistage model because it employed data from each dosage, while yielding low dose linearity and having some vague measure of biological plausibility as cancer is a multistage disease process (Albert, 1994).

[3] The additive to background "concept" is an assumption that was first proposed by Robert Platt (1900–1978), a British physician who specialized in the area of hypertension and kidney function. He was president of the Royal College of Physicians (1957–1962), chairing the committee that published the first major assessment of the Society of Physicians on Smoking and Health, which assembled evidence for a causative relationship with lung cancer (Platt et al., 1962). This publication pre-dated the first report on smoking and lung cancer from the U.S. Surgeon General, which appeared in 1964. The statement of Platt (1955) on the concept of additive to background was a letter to the editor with no reference support. It was a brief commentary written in the form of reasonable but unsupported hypothesis. Perhaps because of his standing in the British medical community and/or the prestige of the journal Lancet, this unsubstantiated concept was adopted by two leading British researchers, Armitage and Doll, in their 1957 presentation of the multistage model of carcinogenesis. Prior to this time, background/spontaneous disease occurrences and/or susceptibilities were generally dealt with under an assumption of independence from background, using Abbott's (1925) correction, a widely adopted methodology in the biological and biomedical sciences, with over 8000 cumulative citations in the Web of Science through 2017.

background multistage dose response model.[4] Armitage and Doll (1957) related the multistage model back to the incidence of cancer in the human population, which was linear when plotting the log of the disease incidence against the log of age. This developmental origin of the additive to background concept would become a cornerstone of low dose linearity as seen in cancer dose-response assessment models.

In the EPA era perhaps the first application of the additive to background concept was published by Albert and Altshuler (1973) in a presentation of the 12th Hanaford Biology Symposium on radionuclide carcinogenesis.[5] Of particular practical significance is that the lead author, Roy Albert, a professor at New York University, would become the director of the newly created unit within EPA called the Carcinogen Assessment Group (CAG) in 1976. Their paper modelled the effects of (1) radium-induced osteosarcoma in CF_1 female mice to a single iv dose of Radium 226 at age 70 days, (2) a three times weekly skin painting of dibenzanthracene (DBA) to mice across four treatments (1.0–9.0 μg) and (3) a smoking and lung mortality study of nearly 300,000 U.S. veterans. The methodology assumed the spontaneous and carcinogen-induced cancers were additive, induced by similar/identical mechanisms with the spontaneous cancers given an equivalent carcinogen dose rate. For example, using model based disease estimates, non-smokers were estimated to receive the equivalent of one cigarette/day while the control subjects in the osteosarcoma mouse study received the equivalent of 0.03 uc/kg radium 226. The effective dose rate is therefore the sum of the applied dose rate and the spontaneous equivalent dose rate. The procedure yields a more conservative risk estimate (i.e., higher tumor incidence) than an independent of background assumption, providing a higher cancer risk at a lower carcinogen dose rate. The Albert and Altshuler (1973) approach, which is based on Druckrey's dose-time response model, assumed that the additional incidence (i.e., treatment effect) had a time to tumor occurrence similar to the spontaneous tumor incidence. However, when the induced tumors were assumed to be independent of background, the induced tumors at low dose rates were predicted to occur after the survival cut off for the experiment, yielding lower overall tumor estimates. The linkage of time to tumor as being the same for spontaneous tumors and induced tumors in the additive to background framework but not in an independent of background approach is a unique but unexplored hypothesis. In the independent of background approach, the tumors appearance would be inversely related to dose and not related to the time of occurrence of spontaneous tumors. In the framework of Albert and Altschuler (1973) this could have considerable impact on carcinogen risk estimates. While the dose-time-response models of Blum et al. (1942), Blum (1959) and Jones and Grendon (1975) would have continuing appeal to Albert, they were not followed by Crump et al. (1976) and received criticism by Hoel, Schneiderman and others during the OSHA (1980) carcinogen hearings.

The additive to background concept would be assessed and refined by others in several influential publications during the mid-late 1970s, including by Crump et al. (1976), soon reinforced by Guess et al. (1977) and then integrated into the range of stochastic, mechanistic, and tolerance distribution models (see Szymczak and Szadkowska-Stanczyk, 2005 for a review). During the mid-1970s, mechanistic understandings of environmental induced tumor/cancer development were very limited as Crump et al. cited only two papers by Knudson (1973, 1974) to support their adoption of the additive to background assumption. A careful reading of these two papers provides no relevant direct support for the additive to background assumption, suggesting that the reviewers may never have assessed the Knudson papers cited by Crump et al. (1976). In a 1977 article in Science Cornfield (1977) noted that the "additivity [to background] assumption is a major one that lacks experimental support". This lack of experimental support for the additive to background assumption was noted by Munro and Krewski (1981) as well as the Food Safety Council (1980) which indicated this assumption "is far from compelling".[6] Despite these concerns and unresolved limitations this assumption became widely supported [e.g., Peto (1978), Zeise et al. (1987), Gaylor (1992, 1997), Crawford and Wilson (1996), Heitzman and Wilson (1997), Hoel (1980, 1997), Pollycove (1997), Crump (1997, 2017), Lovell (2000), Kodell (2001), Kopp-Schneider and Lutz (2001), Beninson (1988), and Wilson (1978, 2000, 2012a)].

The dominance of the independent of background/Abbott's correction in cancer risk assessment would end by the mid 1980s as seen in the EPA cancer risk assessment guidelines (U.S. EPA, 1986). The reasons for this change to an additive to background assumption were related to several factors. Hoel (1980) reported a profound difference in estimated cancer risk using the probit model when the two background assumptions (i.e., independent and additive) were compared. The additive to background assumption was far more conservative. Secondly, Hoel (1980) showed that even when the independent model assumption accounted for 99% of the background influence it was still completely dominated by the additive to background assumption. Thus, all one had to assume was that an additive to background model could account for just 1% of the cancer process to negate the influence of the independent of background assumptions. These two factors would soon lead to the EPA 1986 change (Table 1). These factors are due to the differential manner in which the background factors are mathematically accommodated without biological or mechanistic support.

Krewski et al. (1995) stated that the U.S. EPA (1986) cancer risk assessment guidelines, in effect, restated a major conclusion of the Crump et al. (1976) and Hoel (1980) papers. That is, according to Crump et al. (1976) "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." Complementing this perspective Hoel (1980) noted that; "If even only a small portion of the background is additive then one is in the linear at low dose situation." The EPA (1986) report stated that; "If a carcinogenic agents 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." However, the EPA (1986) document only cited the Crump et al. (1976) paper in justifying the adoption of additive to background.

Wilson (2000) indicated that the multistage model of Armitage and Doll (1957), Crump et al. (1976), Guess et al. (1977) and others, which include the additive to background assumption, depends on the belief that cancers caused by the pollutant and background (i.e., spontaneous) are indistinguishable, and therefore, pollutant and the background act in a similar way. By "indistinguishable" Wilson (2012a) would later state that the spontaneous and induced cancers, "must be biologically indistinguishable and not merely what a pathologist cannot distinguish." Consistent with the view of Wilson (2012a) was the statement of Lovell (2000) that the additive to background assumption "only holds if the mechanisms are exactly the same, not just similar or variants of one

[4] The original model of Armitage and Doll went through a few iterations including incorporating the assumption that the rates of occurrence of the different changes were proportional to dose, thereby permitting the cumulative tumor incidence to be estimated via a simplifying equation. The facilitating procedure was then extended to incorporate a background incidence in the absence of dose (do). These modifications provided a vehicle to assess spontaneous tumor incidence at each stage. A different form of the multi-stage model by Crump et al. (1976) assumed that all carcinogenesis occurred via a common mutagen induced mechanism. The exogenous carcinogen treatment simply enhanced this ongoing process. As noted in the main body of the text, the Crump et al. model assumes that any dosage related treatment effect acts via identical mechanisms as that causing background disease incidence (Lovell and Thomas, 1996).

[5] The BEIR I Committee (NAS/NRC, 1972) qualitatively addressed the issue of background radiation (100 mrem/yr). They recommended keeping the additional radiation dose to the population from human sources (activities) below the normal background quantity. They claimed that this would assure that the additional consequences will neither differ in kind from those which people have experienced throughout human history nor exceed them in quantity. Thus, the natural background was employed as a type of comparison (NAS SDWC, 1977-page 879 for discussion).

[6] Recognition of these limitations likely contributed to the failure of the additive to background assumption being accepted by regulatory agencies during the late 1970s and early 1980s. Furthermore, the long history of Abbott's correction for background led to its initial acceptance by the FDA and EPA.

Table 1
Supportive perspectives of additive to background assumption.

Crawford and Wilson (1996), page 305	"In 1976, Crump, Hoel, Langley, and Peto described how almost any dose-response relationship for carcinogens becomes linear at low doses when background cancers are taken into account. This has been used, by the U.S. Environmental Protection Agency, USEPA, as partial justification for a regulatory posture that assumes low-dose linearity…….The argument depends critically on the assumption that the pollutant and the background proceed by the same biological mechanism."
Crawford and Wilson (1996), pages 305 and 306	"Crump et al. (1976) and Guess, Crump, and Peto (1977) reminded us that when a small amount of pollutant is added to a large amount of the same pollutant, or to another pollutant operating in the same way as the first, a response linear with the incremental dose of pollutant can result. This was a simple and elegant idea that followed mathematically from the fact that the first derivative of a smooth curve is finite. This idea was applied to cancer risks and was used as a partial justification for the US. Environmental Protection Agency's (USEPA) use of low-dose linearity in regulating carcinogens."
Crawford and Wilson (1996), page 324	"A positive, favorable, effect (hormesis) has been suggested at low doses. A biological mechanism (repair) has been suggested to explain why the number is below the line. If there is such a favorable effect at low doses, there is then a nonmonotonic dose-response, and the argument becomes very complex. It would still be important to know whether radiogenic cancers are assumed to result from the same mechanism as naturally occurring cancers. If they do so result, there could be situations where hormesis could be biologically correct in the absence of background, but not true in real situation where the background had already brought the total dose to a region of positive slope of the dose-response curve."
Crump et al. (1976), page 2973, right column	"If the addition of the test carcinogen merely increases the rates of processes that were occurring anyway, then dose-response relationships will be linear at low dose levels……we are chiefly discussing direct carcinogenic processes in which the compound or its metabolite acts at the cellular level to produce an irreversible and heritable (genetic or epigenetic) change."
Crump et al. (1976), page 2977, right column	"Cancers thought to be induced are generally indistinguishable from "spontaneous cancers". This obviously does not demonstrate that the cancers arise by a common mechanism, but it is consistent with a common pathway to "induced" and "spontaneous" carcinogenesis."
Crump et al. (1976), page 2977, right column	"The view of carcinogenesis as a fundamentally mutational phenomenon, as recently reviewed by Knudson (1973, 1974) supports the assumption that induced and spontaneous steps are mechanistically identical." This statement reflects very limited understanding of the process of carcinogenesis. While this perspective may have been widespread during the mid-1970s it has been superseded and replaced with modern molecular advances. The assumption of Crump et al. (1976) lacked adequate evidence and can no longer be used to support the additive to background assumption employed in low dose linearity.
Crump et al. (1976), page 2977, right column	"Small extra doses of a carcinogen will therefore elicit linear increases in risk for virtually any response model."
Crump et al. (1976), page 2978, left column	"Virtually all models of carcinogenesis that depict the exposure as affecting an already ongoing process will lead to linearity at low dose."
Crump et al. (1976), page 2978, left column	"….this assumption of dependence or common mechanism is not trivial. It can make orders of magnitude difference in the estimated risk associated with low dose exposure."
Crump (1997), BELLE Newsletter	"Based on the additivity to background argument, in order to justify a biologically–based model of underlying mechanisms that predicts a non-linear low-dose response, it is necessary to rule out completely the possibility that non-pollutant-related responses could ever occur via a common mechanism. This would appear to be a difficult task in many, perhaps most, situations. The argument also suggests that the shape of the dose response curve at low doseis inherently determined by the relationship between background responses and responses induced by the pollutant. This idea seems to have been largely ignored by researchers in designing and interpreting experiments with the goal of understanding low dose risk. Perhaps a fruitful line of research would be to develop data that could be used to better understand (and perhaps refute) the additivity to background argument for low-dose additivity."
Heitzmann and Wilson (1997), page 2	Crump et al. (1976) and Guess, Crump, and Peto (1977) pointed out that if a pollutant produces cancer via the same mechanism by which background cancers occur, then there results a linear incremental response to the incremental dose. Even if the biological response mechanism has a threshold, or is non linear, the existence of the background cancers shows that the threshold is already exceeded by a background pollutant. Then when a small amount of the same pollutant, or to another pollutant operation in the same way as the first, is added, an incremental response linear with the incremental dose of pollutant can result, almost independently of the particular biological mechanism relating dose and response. This simple and elegant argument follows mathematically from the fact that the first derivative of a smooth curve is always finite."
Heitzman and Wilson (1997), page 7	"….it is important not only measure the magnitude of the biological dose-response, but also to have some understanding of the underlying biological mechanism(s) in particular, whether the mechanisms are the same for pollutant- and background-induced effects. It is also clear that if the background and the pollutant effect operate by the same mechanism, the slope of the dose-response function at low doses can be much lower than the slope at high does if he background itself is small."
OSHA (1980), Carcinogen Hearings, page 5185, right column - Federal Register 45(15):5185 and 5186 (Peto/Rall)	"Mr. Richard Peto (Oxford Univ.) explained that the most models predict a linear dose-response relationship at low dose, because of the probable existence of "equivalent background", i.e., exposure to other background carcinogens to which the effects of an added carcinogenic agent will be linearly additive (Peto, S.1: Annex B; Peto 1978). He explained during his oral testimony: Now, if we admit the possibility of such a background, then it turns out that all the arguments as to whether we should have linear models, K-hit model, one-hit models, probit model, log normal models or whatever, becomes irrevelant to the mathematical estimation of the upper confidence limit on the risk associated with a particular low dose. If any of these models is formulated in the mathematical form that the risk is equal to some function—probit, log normal, whatever- of dose plus background dose, then the consequences will be the same. Where background dose now becomes a parameter to be estimated statistically and we always finish up with linear upper confidence limits; you know, that the risk just turns out to be proportional to the dose at low doses." (Peto, Tr. 2514)."
OSHA (1980), Carcinogen Hearings, page 5185, right column - Federal Register 45(15):5185 and 5186 (continued)	"Essentially the same point was made in detail in the original paper by Peto, Hoel and their colleagues in which the multi-stage model was developed (see comment by Hoel quoted above):

(*continued on next page*)

Table 1 (*continued*)

	Virtually all models of carcinogenesis that depict the exposure as affecting an already ongoing process will lead to linearity at low dose. We have discussed the validity of this assumption above. This result then implies that, no matter what the biological mechanism we might imagine, if the carcinogen increases some part of the already ongoing process, then we should expect the response to be approximately linear at low dose. As pointed out above, this assumption of dependence or common mechanisms is not trivial. It can make orders of magnitude differences in the estimated risk associated with low dose exposure."
OSHA (1980), Carcinogen Hearings, page 5185, right column; page 5186, left column	"…if we conceive of the cell alteration process as a series of discrete single-cellular random events that can occur in sequence or in any given cell and that a dose-independent induction period follows, then we should expect dose-response over background to be linear. We have required neither that all steps be affected by the carcinogen (only some) nor that these steps be all mechanistically similar in quantity or quality. This general class incorporates most of the reasonable models that have been proposed. The keys to this result are the assumptions of the single-cell origin and the lack of any appreciable dose dependence in the induction period."
OSHA (1980), Carcinogen Hearings, page 5185, left column	"A further extension of the group of models allows the incorporation of threshold models into the class of "linear at low dose." We have indicated that, if we conceive of single cells as the biological unit at risk and that the initiation response is a threshold phenomenon, then by assuming that the threshold is randomly distributed in dose we find that the low dose response of the whole tissue over background will be approximately linear. If, rather implausibly, we do suppose that some sort of cellular thresholds exist, then clearly all cells do not have the same threshold since all cells do not all become cancers simultaneously. Here again, we have assumed that the carcinogen acts in conjunction with the "spontaneous" or background effects."
OSHA (1980), Carcinogen Hearings, page 5136, left column	Mr. Richard Peto (Oxford Univ.) developed this point at some length in his direct statement (S. 5-10 and Annex B) and presented a mathematical proof that under rather general conditions no threshold would be expected. The basic requirement for this proof is the existence of "carcinogenic background alternatives", i.e., the existence of other agents in the environment of the exposed animal which give rise to the same functional effect: The second assumption needed is that some carcinogenic background alternatives exist to the carcinogen being considered, i.e., *some* of the spontaneous cancers that arise do so by mechanisms which are functionally similar to those whereby the test carcinogen works. Again, I believe that for *most* carcinogens *some* carcinogenic background alternatives are likely to exist, although again I would expect a few counter-examples to occur. (The counter-examples would probably involve cases where the carcinogen produces some gross pathological effects, such as suppression of ovulation, which then affects cancer risk, rather than cases where it acts directly by causing a local DNA lesions.)"(Peto, S. 6). In introducing this section of his testimony, Mr. Peto explained that most arguments for the hypothesis of thresholds or extremely low risks associated with low doses are due to failure to recognize the possibility that "equivalent background" may exist. Accordingly, he referred to the concept of thresholds as "nonsense". (Peto, Tr. 2515)."
OSHA (1980), Carcinogen Hearings, page 5136, left column	"Dr. David Rall (Director, NIEHS) made essentially the same point, both in his opening statement (Rall, Tr. 354) and in response to a questions about protective mechanisms: …I think however, it becomes almost ridiculous when you recognize that 16% of the people in this room are going to die of cancer. I mean, this talk of a threshold is, to me, sort of an ethereal dream world. You know, 16% are going to die of cancer and about twice that will get cancer in their lifetime. I am sorry, but I think the threshold was exceeded a long time ago, and I just do not see any point in talking about it anymore, in terms of the population."(Rall, Tr. 397-398).
Wilson (2000), page 297, left column	"Crump et al. (1976) and Guess et al. (1977) pointed out that the argument for low-dose linearity is far more general than the Doll-Armitage theory and depends solely on the fact that cancers caused by the pollutant and background are indistinguishable, and therefore it is likely that the pollutant and the background act in a similar way." "These analyses were used by the U.S. Environmental Protection Agency (EPA) 25 years ago as a justification for assuming low-dose linearity as a general default."
Wilson (2012b), page 481-482	"…….the usual (conservative) model suggests low dose linearity. This comes from the realization that if a medical outcome of a pollutant or action is indistinguishable from one that occurs naturally, any addition to natural incidence is proportional to the dose at low doses (Crump et al., 1976; Guess et al., 1977; Crawford and Wilson, 1996). Indeed, this is also a consequence of the usual application of the multistage theory of cancer as described over 50 years ago (Armitage and Doll. 1954)." "….there are assumptions and approximations. In the justification I used the word "indistinguishable and not merely that a pathologist cannot distinguish."
Wilson (2012), page 2014, right column	"Low-dose linearity follows from a general argument enunciated by Guess et al (1977) and Crump et al (1976), which follows from Taylor's theorem (Hazewinkel, 2001). If a medical outcome caused by a pollutant is indistinguishable from an outcome that can occur naturally any small increase in dose is likely to lead to a corresponding small increase in effect (Crawford et al., 1996)."
Zeise et al. (1987), page 263, left column	"For very rare cancers, the effective background dose dB may be practically zero and the shape of the dose-response curve indistinguishable from that expected under the assumption of dose independence. On the other hand, if the cancer in question is common, with dB relatively large, the response at low doses of the external agent would be linear."
Zeise et al. (1987), page 263, left column	Commenting on a Krewski and Van Ryzin paper, they stated that "when the observed background doses were estimated and extrapolations down to an excess risk of 10^{-5} were performed and when the observed background tumor incidence was greater than about 1%, the predicted response at low dose was essentially linear in dose. However, "for background tumor incidence less than 1%, low dose linearity did not result when dose-response formula allowed for curvature, and the high dose data exhibited nonlinearity."
Zeise et al. (1987), page 262, right column	"An additive assumption: The spontaneous cancers are produced by the same mechanisms as the cancers produced by the xenobiotic…….this always gives a response linear in the external dose at sufficiently low doses if the first derivative of F is finite……The idea that the carcinogen may add to an effective background dose has been often used to support the claim that a linear dose-response formula is never overly conservative."

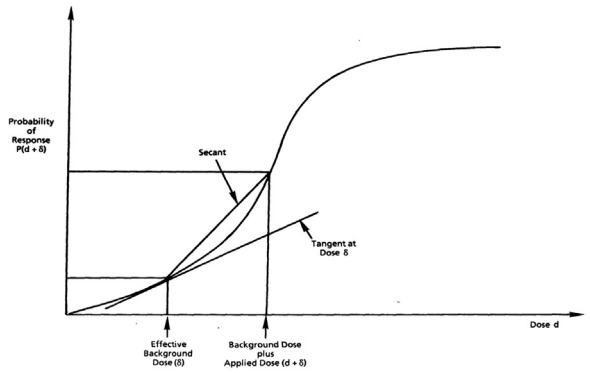

Fig. 1. The additive to background model (source: U.S. EPA, 1989).

another". As will be subsequently shown, this hypothesis became experimentally testable only well over a decade after Crump's et al. (1976) original formulation.

3.2. The additive to background rationale

Using the argument of Crump et al. (1976), Wilson and colleagues (Crawford and Wilson, 1996; Wilson, 1997) thought it reasonable to assume that most biological responses would be non-linear, but monotonically increasing. In a situation of zero background disease incidence, the dose-response relationship at low doses would reflect an obvious non-linearity as an infinitesimal dose would yield very slight (i.e., negligible) increase in response. Thus, this scheme assumes the existence of an "equivalent background dose" (do), that would create the background response. The general requirement for the additive to background based model demands that the toxicologically based dose response curve be smooth and monotonical while the background agent and toxic agents (i.e., pollutants) must act via the same mechanism(s) (Zeise et al., 1987; Krewski et al., 1995; Wilson, 1996, 1997). As noted by Krewski et al. (1995) and Rhomberg et al. (2011) the enhanced/extra risk over background is linear at low dose with the incremental/positive response over background being approximated by the tangent to the curve at zero dose (Fig. 1). As noted by EPA (1989) the spontaneous tumor rate increases as a result of an effective "background dose" the effects of which act additively to background via a dose related manner. The excess risk over background, which is linear at low doses, occurs due to the fact that the secant between doses of δ and (δ + d) converges to the tangent of the dose response curve when the dose d of the agent under evaluation becomes small. This situation creates a theoretical framework in which the unexposed population is, as noted by Rhomberg et al. (2011), already to some extent up the response curve at zero exposure to the agent under study/evaluation (Fig. 1). This would be the case even if the dose response mechanism for carcinogens acted via a threshold, linearizing the dose response for such agents (Zeise et al., 1987; Wilson (1997); Fisher (1984)). The general view was that humans display a relatively high cancer background incidence in the 20–25% range over a lifetime. New exposures to chemical/radiation-carcinogens were assumed to directly add to this background burden, resulting in an increased incidence of cancer and/or its accelerated appearance (i.e., shorter latency period) (Platt, 1955). This view was reinforced by Gaylor (1997) who noted that the most compelling argument supporting low dose linearity is the "situation where a chemical augments a background tumorigenic process… The presence of background tumors indicates that existing endogenous and/or exogenous factors already surpassed a threshold dose, if one exists".

When a chemical is designated as a human or probable human carcinogen and of regulatory concern, it may be evaluated further via the application of quantitative risk assessment. Of particular significance in this process is the derivation of the cancer slope factor,

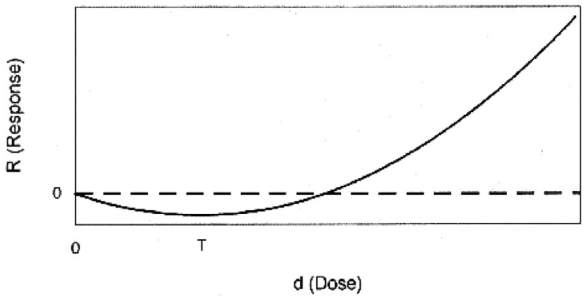

Fig. 2. Hormetic dose response: Additive to background low dose linearity response starts at the nadir (optimal treatment = T) of the hormetic (adapted from Crump, 1997).

which quantitatively defines the relationship between the dose and response. According to the EPA the cancer slope factor represents a plausible upper-bound estimate of the probability that an individual will develop cancer if exposed to a chemical for a lifetime of 70 years. The cancer dose-response assessment procedure determines a point of departure, which is typically the derivation of the lowest effective dose (LED) for a 10% treatment-related response corresponding to the lower 95% limit of the dose. This value, which is associated with the 10% risk adjusted for background (Wiltse and Dellarco, 2000), provides the practical significance of the additive to background with EPA risk assessment practices.

Krewski and van Ryzin (1981) showed that the additive to background assumption for multiple data sets revealed that when the background disease incidence > 1% the estimated risk at low dose was linear. When the background tumor incidence < 1%, low dose linearity did not occur. Thus, if the cancer under study is common, the predicted response at low dose would be linear. Since a large proportion of human cancers (i.e., breast, ovary, thyroid, colorectal, kidney-pelvic, leukemia, liver and bile duct, lung and bronchi, melanoma, non-Hodgkins lymphoma, oral cavity, pharynges, pancreas, prostate, stomach, and urinary bladder) (U.S. NCI 2011–2013 U.S. statistics) exceed the 1% background tumor incidence, the additive to background assumption would remain operational. An extensive follow up evaluation by Gaylor (1992) was supportive of the general perspectives offered by Krewski and van Ryzin (1981). For linearity at low dose to be dropped from the cancer risk assessment lexicon, the additive to background concept would have to be shown to be incorrect[7] or the hormesis model (Calabrese, 2008; Calabrese and Baldwin, 2003) would have to be adopted. In the case of hormesis, if additive to background were accepted, the response would start at the nadir of the J-shape dose-response, where the cancer risk is less than background creating, in effect,

[7] Hoel (1980) provided a biostatistical estimate of the impact of a mixture of independent and additive to background modeling for cancer risk assessments using the probit model. The exercise revealed that the additive to background assumption strongly dominated the risk estimated even when 99% of the sample was assumed to be independent. He indicated that even if additive to background was a minor factor it would still, in effect, drive the risk assessment to linearity at low dose. He stated that for the independent of background condition to have an important impact on the cancer risk it must be 100%, even 99% independent would not be influential. The assessment of Hoel was limited to assume a slope of 1 using the probit model. Whether the findings of Hoel (1980) would be generalizable to other slopes and/or models was not addressed. Yet, it was used by EPA (1986) as justification to continue its acceptance of the additive to background assumption in cancer risk assessment. According to Bogen (January 5, 2018-personal e-mail communication to Edward J. Calabrese) the argument of Hoel is independent of model and slope when the background response is non-zero. Despite such theoretical comments, Harris Fischer (1984) noted that "we will determine whether background acts additively or independently not by contemplation of dose-response relationships, but by whether we find such mechanisms at work in biological experiments. Presumably, this is something that we can eventually get a handle on by looking at the actual microbiological processes occurring within the DNA molecule." This comment by Fischer nearly 35 years ago anticipated the oncogene revolution and the intent of the present paper to evaluate the additive to background hypothesis with modern molecular biological mechanisms.

Table 2
Critical perspectives on additive to background assumption.

Heitzman and Wilson (1997), page 6	"The usual assumption that background- and pollutant-induced biological effects proceed by the same biological mechanism may not be correct in the case of benzene. There is some evidence that benzene-induced leukemiasare always preceded by pancytopenia, which does not occur naturally in the population to any appreciable extent. If this is the case, background leukemias must be produced by an entirely different mechanism than benzene-induced leukemias, and the Crump et al. (1976) argument does not apply."
Davel G. Hoel (1997), BELLE Newsletter	"As one of the original authors (of the Crump et al., 1976) of the low-dose linearity paper, I am less enthusiastic than Crawford and Wilson are over its usefulness. The original paper was written some twenty years ago without benefit of much of today's biology. What is of concern is whether or not the original simple idea of background additivity is consistent with today's biology and whether the concept, if true, has any value for quantitative risk estimation."
Robert L. Sielken Jr. (1997)	He focused on the additive to background mechanism assumption: "First, the background dose and the "pollutant" are assumed to have exactly the same mechanism of action (that is, that the background dose and the pollutant dose are additive in the dose-response relationship). For example this means not just that the background dose and pollutant both cause liver tumors, but also that they both affect the same cell types, affect the same stage in a multiple stage process, and cause the same type of cellular activity (e.g. both cause the same adduct, both inhibit the same repair processes, or both impact cell proliferation or apoptosis in the same way).
James D. Wilson (1997)	This is a critique of the Heitzman and Wilson 1997: "…their conclusion rests on a mathematical analysis of a special case and a few examples, one an obvious and acknowledged exception. For science, the proof of their proposition is not persuasive. …. They and Crump et al. argue that the dose response is continuous and monotonically increasing. A relation that is discontinuous, a step function, for example, will obviously not produce a straight line when added to a background process. The result will still be a step function…..argues that the rate limiting step consists of induced proliferation in cells in the target organ, a process that is very tightly controlled, physiologically.

a dose response threshold (Fig. 2) (Crump, 1997).

3.3. The additive to background assumption and molecular mechanisms: How does this assumption hold up in the modern era

3.3.1. Early questions, concerns, and affirmations of additive to background

About two decades after the Crump et al. (1976) article on additive to background, Hoel (1997), a co-author of that paper, wrote **"what is of concern is whether or not the original simple idea of background additivity is consistent with today's biology and whether the concept, if true, has any value for quantitative risk estimation"**. According to Hoel (1997), troubling inconsistencies in the original additive to background concept as applied to risk assessment have been reported, such as that different carcinogens produce various cancerous subtypes. For example, cigarette smoking induces an acute myelogenous leukemia subtype M2, while benzene has a high relative risk for subtype M4. Moreover, other acute myelogenous leukemic subtypes are associated with ionizing radiation.

In the case of acute leukemia Taylor et al. (1992) found 15% had activated RAS oncogenes. While exposure to solvents has been associated with increased risk of RAS-activated acute leukemia it has not been associated with non-RAS acute leukemia. These complexities indicate that acute leukemia in humans displays multiple possible pathways and raises the likelihood that an agent may affect only a specific pathway. Based on the above findings, Hoel (1997) suggested that the existence of multiple pathways might affect the additive to background argument.

Hoel (1980) also challenged the additive to background concept for a cell proliferation tumor promotion mechanism, stating that since cells divide spontaneously, this assumption should apply. However, this was a position he found unpersuasive. This later criticism is important as it complements the principal focus of mechanisms inherently genetic/oncogene related. Table 2 provides a range of historical perspectives by leading experts that are supportive and critical of the additive to background assumption.

The additive to background features of the cancer dose-response assessment process were based on an assumption that was not verified nor experimentally explored prior to incorporating it into EPA and other regulatory agency risk assessment principles and practices. The molecular tools simply did not exist at that time to test the underlying hypothesis. It was also an assumption/belief that failed to become the object of an adequate timely follow-up review after its adoption by EPA.[8,9] It simply became the equivalent of a "codified" assumption, a "basic tenet", accepted without an adequate assessment and factual basis.

3.4. Testing the additive to background hypothesis

Contradicting the additive to background assumption, numerous papers have demonstrated that spontaneous tumors often are initiated and progress via different mechanisms (Table 3) than chemically/radiation-induced tumors of the same organ (e.g., hepatocellular carcinomas, lung tumors, forestomach tumors, Harderian gland tumors). This is the case despite similarities at the level of tumor location and histopathological evaluations (Table 4). The principal comparison of

[8] In a March 15, 1979 notice in the Federal Register, Douglas Costle, the EPA Administrator, indicated that the single-hit model "has been modified to account for spontaneous tumor incidence". An Appendix to this statement by Administrator Costle indicated it incorporated the concept of spontaneous control group tumor incidence via an independent of background assumption using Abbott's correction (Abbott, 1925), which was incorporated into the one-hit model. Abbott's correction was subsequently incorporated in the multi-stage model after its adoption by EPA in November 1980 as reported by Anderson (1983).

[9] In 1989 the EPA conducted a workshop on various aspects of carcinogen risk assessment. One aspect included the additive to background assumption. The additive to background assumption session activities were summarized in the workshop proceedings. While the conclusions reaffirmed past assumptions and practices, the workshop identified a series of key questions that guided and/or emerged from the workshop activities. The hypothetical questions posed were:

a. Is the additive-to-background position an assumption or are there data to suggest that it describes the underling biological truth?
b. How does the statistical argument that low-dose linearity is to be expected when mechanism is additive to background fare in view of knowledge of various mechanisms of carcinogenesis?
c. How can we distinguish cases of independent and additive background in practice?
d. What biological data can help in trying to make this distinction? What is known about the low-dose properties of dose-effect curves for elements of proposed mechanisms of carcinogenesis (e.g., mutation, cytotoxicity, receptor binding)?
e. Practically speaking, are we able to measure very small elevations in these processes over background so small that they imply trivial cancer consequences in order to detect a virtual (or practical) threshold?
f. For quantitative purposes, should a putative epigenetic carcinogen be treated as acting independently from or additively to low levels of other such agents in the human environment? Of genotoxic agents in the human environment?
g. In view of the above issues, under what circumstances might it be appropriate to assume that carcinogenesis has or does not have a dose threshold?
h. What criteria must be satisfied to treat a carcinogen as acting independently from background, and how should exposures to these substances be viewed vis-a-vis exposures to substances that may be additive to background?

Table 3
Mechanism comparison of spontaneous vs induced tumors: Assessment of additive to background assumption.

Citation	Comparison Endpoint	Agent	Animal Model	Tumor	Evaluation/Comment
Antal et al. (2002)	Loss of heterogenecity (LOH)	Gamma irradiation	C57BL/6 female DBA2 male mice	Liver and lung tumors	LOH at D4Mitt77 (liver) and Acrb (lung) were identified in treated mice but not in spontaneous tumors.
Blackshear et al. (2015)	Gene upregulation	Vinylidene chloride (VDC)	F344/N male rats	Peritonael *meso*thelioma	Mesotheliomas from VDC-exposed rats ALONE displayed an over-representation of pro-inflammatory pathways and immune dysfunction including nuclear factor kappa chain-enhancer of activated B cells signaling pathway, IL-8, Il-signaling, interleukin responses, Fc receptor signaling and natural killer and dendritic cells signaling pathway, presentation of DNA damage and repair. (abstract) Mesotheliomas from VDC-exposed rats displayed upregulation of genes with tissue damage (Tlr2, Dpt, Mrcl, Pla2g2a) and damage-associated molecular pattern (DAMP) molecules (S100a*, S100a9, Mc1, and Lyvel). (page 176, right column) Mesotheliomas in all VDC-exposed rats were morphologically similar to spontaneous *meso*theliomas in F344N male rats. In many cases the expression of these genes were "fairly" similar between spontaneous and VDC *meso*thelioma, both displayed an overlap of genes associated with multiple molecular categories. (page 176, left column and page 1606, left column)
Chen et al. (1993)	Oncogene comparison	NDEA; NNK	B6C3F1 mouse	Liver tumor; lung tumor	Liver (NDEA): the mutation spectrum of activated H-ras genes was different than that reported for spontaneous mouse liver tumors. This was based on the proportion of mutations at the three locations of Codon 61 (CAA→AAA, CGA and CTA of the H-ras). There was also a dose dependent difference in the mutation spectra. This suggests that high doses do not predict responses at low doses well. Lung (NNK): As in the case with the liver, there was a significant difference in the specific location of activated K-ras for Codons 12 & 61 between the spontaneous and NNK treated mice. There also was a dose dependency issue for lung tumor oncogene responses as well.
Chinsky et al. (1985)	Location of tumor development	Methylcholanthrene CMA	RF/J mice; AKR mice	Thymic lymphomas	"…T-lymphocytes comprising tumors induced by MCA differ from the T-lymphocytes in spontaneous AKR tumors in the frequency with which they express c-*myc* and c-*myb* transcripts." (page 568, left column). Presented "evidence suggesting that thymic lymphomas induced in RF mice by skin painting with MCA differ significantly from spontaneous thymic lymphomas of AKR mice." (page 568, left column) "Spontaneous thymomas develop in thymic medulla while the MCA induced thymomas occur in the cortex. This happens in both models." (page 568, left column)
Devereux et al. (1991)	Oncogene comparison	NNK; NDMA	C3H mouse	Lung tumor	The activating mutation in all the pulmonary tumors from the NNK or NDMA-treated mice was a GC- > AT transition, located at the second base of codon 12 of the K-ras gene. However, K-ras activation was only reported in 3 of 7 spontaneous lung tumors: one GG to TA, (2nd base of codon 12), one GC to AT transition in the 2nd base of codon 12, and one AT to TA in the 3rd base of codon 61. (page 301, right column)
Donnelly et al. (1996)	Oncogene comparison	Aflatoxin B	AC3F1 (A/J x C3H/HeJ) mice	Lung tumor	All of the lung tumors from AFB1 treatment displayed K-ras codon 12/13 (exon 1) mutations at quanine residue while 65% of K-ras in the spontaneous tumors were located in codon 61 (exon 2) with mutations at the quanine and adenine residues. (page 1738, left column Nearly all lung tumors in AF1-treated mice had K-ras codon 12 mutations with most being at GTT followed by GAT and TGT. The controls showed a markedly different pattern with few mutations to GTT and TGT. (page 1738, left column)
Fox et al. (1990)	Oncogene comparison	Benzidene; Phenobarbital; chloroform; coprofibrate	C57B1/6 x C3H/He	Liver tumor	Liver tumors induced by these carcinogens had much lower frequency of H-ras activated than spontaneous liver tumors. These were also related to codon specificity.
Hayashi et al. (2001)	Oncogene comparison	1-amino-2,4-dibromoanthraquinone (ADBAQ)	B6C3F1	Forestomach tumor	Predominant H-ras codon 61 CAA- > CTA transversions were detected in ADBAQ-induced; these were not considered to be spontaneous because such mutations have not been detected in spontaneous forestomach tumors examined to date in B6C3F1 mice.
Hong et al. (2003)	Oncogene comparison	o-nitrotoluene; riddelliine	B6C3F1	Hemangiosarcomas (HS); skeletal muscle tumors; mesentery tumors	There was a lack of ras, p53 and B-catenin mutations in the spontaneous HS. It provides a foundation to compare with carcinogen-induced HS. A high proportion of K-ras codon 12 G to T transitions occurred in riddelliine-induced liver HS transversions. In controls, ras mutations were detected with o-nitrotoluene treatment but at a lower frequency. However, it showed high frequencies of B-catenin mutations. Both agents showed high incidence of p53 mutations. (page 233, top left column)

(*continued on next page*)

Table 3 (*continued*)

Citation	Comparison Endpoint	Agent	Animal Model	Tumor	Evaluation/Comment
Hong et al. (2007)	Oncogene comparison	Ethylene oxide	B6C3F1	Lung tumors; Harderian gland tumors	The pattern of K-ras oncogene activation was significantly different between the spontaneous tumors of the controls and the tumors of the treated groups for Harderian gland neoplasms and lung tumors. There also appears to be a significant reprogramming within control groups for the lung and the Harderian gland tumors.
Hong et al. (2008)	Oncogene comparison	Comene	B6C3F1	Lung tumor	The comene treated mice displayed a markedly different mutation spectra than the controls across codons 12, 13, and 61. Major changes in the related frequency of codon mutations were seen in codon 12 (GAT and TT or codon 61 (CGA and CAT)
Hong et al. (2015)	Oncogene comparison	Cobalt metal	B6C3F1/N	Lung tumor alveolar/ bronchiolar carcinoma	The incidence of K-ras mutations in lung tumors from mice chemically exposed to cobalt was 67%. The majority of K-ras mutations were localized within codon 12 (43%) (30/69) followed by codon 61 (20%) and codon 13 (6%). The most common codon 12 mutations in lung tumors from all cobalt exposure dose groups was a G to T transversion.
Houle et al. (2006)	Oncogene comparison	Benzene; ethylene oxide	B6C3F1	Mammary gland carcinomas	The mutation spectra of H-ras codon 61 and P53 for codons revealed significant difference between the tumors of the controls and treated mice. Noteable suppression of control group oncogene activation was reported for both the H-ras and P53 codons.
Iizuka et al. (2010)	Chromosome aberrations	Gamma Ray	Sprague-Dawley	Mammary carcinomas	Characterized genomic copy number aberrations for radiation induced rat mammary carcinomas. Reported 14 carcinomas (2 Gy dose) and 26 aberrations, including trisomes of chromosomes 4 and 10 for three and one carcinomas, respectively, and an amplification of the chromosome region 1q12 in two carcinomas, and deletions of the chromosomal region 3q35q36, 5q32 and 7q11 in two, two and four carcinoma. These were not found in spontaneous mammary carcinomas. First report of array CGH (comparative genomic hybridization) analysis for radiation-induced mammary tumors showing distinct aberration patterns, different than spontaneous tumors. (abstract)
Imaoka et al. (2008)	Gene expression	Radiation Gamma-rays	Sprague-Dawley rats	Mammary Carcinomas	Gene expression analysis distinguished between spontaneous and radiogenic carcinomas, suggesting possible differences in their carcinogenic mechanisms. The data indicate that spontaneous and radiogenic mammary cancer developments involve distinct molecular and cellular mechanisms. 50 genes were identified that displayed different expression levels between spontaneous and radiogenic carcinomas. (abstract)
Kawano et al. (1996)	Oncogene comparison	NNK	A/J female mice	Lung tumors	K-ras gene mutations were found in 72.2% of all NNK-induced lung lesions; the principal mutations was a G to A transition at the 2nd base of codon 12. According to the authors the gene mutational change is “clearly different from the mutational pattern in untreated control mice.” However, whether and how NNK induced mutations affect the process of carcinogenesis needs further clarification.
Liu et al. (2003)	Gene deletion comparison	ENU; Gamma Rays	HL-60 cells	Leukemia	The spectra of spontaneous mutations were notably different from that induced by ENU and radiation. Total exon deletion did not occur in a spontaneous mutation, but did so in gamma rays and ENU-induced mutations. The proportion of deletion mutations were very different between the treated and spontaneous mutation groups. Likewise, this was also the case for induced mutations at the hprt locus.
Loktionov (1991)	Oncogene comparison	DMBA	CD-1 mice	Liver tumors	During transplacental carcinogenesis studies DMBA-induced lung tumors were associated with A to T transversions in codon 61, (position two) of H-ras. These mutations were not observed in the liver tumors of controls.
Manam et al. (1992)	Oncogene comparison	DMBA	CD-1 mice	Lung tumors	DMBA induced several types of K-ras mutations not found in spontaneous lung tumors in CD-1 mice. See codons 12, 13, and 61.
Manam et al. (1995)	Oncogene comparison	4-aminoazobenzene; N-OH-AAF; DEN; DMBA	CD-1 mouse	Liver tumors	Spontaneous liver tumors had a low frequency of ras mutations, all of which were in the Ha-ras codon 61, with the majority of mutations (~80%) C - > A mutations – only detectable after transfection. The pattern of ras mutations in AAB-, N-OH-AAF and DEN induced tumors were significantly different than spontaneous tumors. For example, 10 Ki-ras codon 12 (G-C) mutations were found in 37 single dose AAB-induced tumors. The number and distribution of mutation types were significantly different by dose, similar to other studies noted in this review. Multi-dose DMDA-induced liver tumors had A- > T mutations in the Ha-ras codon 61 and G- > C mutations in Ki-ras codon 13. These were similar to single dose responses. The DMBA treatment also had a high proportion of tumors with Ha-ras-codon 12 (G- > A) mutations. The Ha-ras codon 61 A- > T, Ha- and Ki-ras codon 13 G- > C and N-ras codon 12 and 13 G- > T mutations were believed to be induced by the initiating dose since they were not found in the spontaneous tumors.

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Table 3 (*continued*)

Citation	Comparison Endpoint	Agent	Animal Model	Tumor	Evaluation/Comment
Manjanatha et al. (2017)	Oncogene comparison	Ethylene oxide	Big Blue mouse	Lung tumors	The authors reported significant mutation spectra shift at the 200 ppm concentration of ethylene oxide at the 8 and 12 week time period.
Marxfield et al., 2006	Gene profiling	DMBA	Sprague Dawley	Mammary adenocarcinomas	The histologically similar spontaneous and DMBA-induced adenocarcinomas could be separated by gene profiling.
Mass et al. (1996)	Oncogene comparison	Benzo(b)fluoranthene	A/J mice	Lung tumors	Nearly 90% of chemically-induced tumors had a mutation in codon 12, the K-ras oncogene. These mutations had G- > T transversions in the first or second base on codon 12. In controls, the spontaneous tumors had a markedly different pattern GGT- > GAT (61%).
Melnikova et al. (2004)	Genomic alterations	DMBA	Murine, melanoma, cell line B16Fo	Melanoma	Inactivation of the $pl9^{Arf}$ and $P16^{INK4a}$ genes cooperating in the development of spontaneous mouse melanomas, whereas loss of $P19^{Arf}$ or p53 tumor suppressors cooperate with ras and/or mAPK cascade to induce malignant transformation in carcinogen-induced melanomas.
Mittelstaedt et al. (1999)	Oncogene comparison	2-AAF	Hprt; lymphocytes in F334 rats	Lymphocytes studied	Different sequence specificity of Hprt lymphocyte mutation than controls. 2-AAF most common mutation was G:C- > T:A transversion (32% of all mutations); followed by 1-base pair (bp) deletion (19%); there were very few (5%) G:C- > A: transversions, but no 1 bp deletions.
Nesnow et al. (1995)	Oncogene comparison	Benzo(a)pyrene; benzo(b) fluoranthene; cyclopental(cd) pythene; dibenz(a,h)anthracene; 5-methylchrysene	A/J mouse	Lung tumor	A treatment-induced shift in tumor mutation frequency and in the mutation spectra. For example, in the spontaneous A/J mouse the codon 12 GAT mutation was reported to occur in 59% of tumors while codon 12 TGT initiation did not occur. In contrast, in the BAP treated mice the codon 12 GAT mutation occurred in only 19% of tumors and the codon 12 TGT mutation was observed in 50% of tumors.
Nishimura et al. (1999)	Oncogene comparison	Gamma-Rays	Scid mice	Thymic lymphomas	Neither activated K-ras or N-ras were found in the spontaneous lymphomas. However, K-ras mutations increased in a dose-dependent fashion in the radiation-induced lymphomas. Analysis of the spectrum of K-ras mutations revealed unique mutations in both codon 13 (GGC- > GAC) and codon 61 (CCA- > CTA) in addition to the more commonly observed substitutions of GAT- > GGT in codon 12 of K-ras.
NTP Tech Report 507 (2002)	Oncogene comparison	Vanadium pentoxide	B6C3F1 mouse	Lung tumor	Major mutation profile differences occurred with K-ras 12 codon; this is seen with GTT, TGT, and to a lesser extent with GAT mutations.
Reynolds et al. (1986)	Oncogene comparison	Liver carcinogens (not specifically identified)	B6C3F1 mouse	Liver tumors	"At present, any relationship between spontaneous tumors and the high frequency of chemically induced tumors in the liver of the B6C3fF Mouse is **unclear**." (p36- Column #2).
Reynolds et al. (1987)	Oncogene comparison	Furan and furfural	B6C3F1 mouse	Liver tumor	These two agents tested positive for tumor induction in the mouse liver 2-year carcinogenicity studies, but test NEGATIVE for induction of mutation in Salmonella assays. "The spectrum of activating mutations in the H-ras gene and the pattern of ras gene activation observed in the chemically-induced liver tumors differ significantly from those observed in the liver tumors of untreated animals. All of the activated ras genes detected in the spontaneous liver tumors were H-ras with activation by point mutations at Codon 61. However, only 60% and 40% of the ras oncogenes detected in the furan and furfural, respectively, were activated K-ras or H-ras genes with mutations and positions other than codon 61."
Reynolds et al. (1988)	Oncogene comparison		BC3F1 mouse	Liver tumors	"Activated Ki-ras genes were detailed in the chemically induced liver tumors but not in the spontaneous liver tumors." (refs. 9 & 10). "…all of the H-ras oncogenes detected in spontaneous liver tumors were activated by point mutations in codon 61 whereas H-ras oncogenes detected in the chemically induced tumors were activated by point mutations at a number of different codons." (see reference 10). In the F344/N rat strain of the NTP program with 20 benign spontaneous lung tumors there were no detectable oncogenes. However, 18 of 19 chemically induced lung tumors had detectable oncogenes in K-ras codon 12.
Rumsby et al. (1991)	Oncogene comparison	DEN; Phenobarbitone	C3H/He mice	Liver tumors	This study demonstrated a striking difference between tumors induced by DEN or PB and those that occur "spontaneously" in C3H/He mice. Codon 61 mutations were reported in 41% of DEN induced tumors (19/41), with either first base (CG- > AT, 12/19), a transversion, or the second base (AT- > GC, 7/19) a transition. Codon 61 mutations (all CG- > AT 6/21) were detected in 29% of spontaneous tumors. However, none were found in the PB induced tumors (0/15) and in normal tissue of untreated mice (0/30).
Sills et al. (1995)	Oncogene comparison	Ozone; methylene chloride; tetranitromethane, 1,3-butadiene	B6C3F1 mice	Lung tumors	The control group (concurrent) displayed a markedly different K-ras mutation pattern than ozone, MC, tetranitromethane and 1,3-butadiene for codons 12, 13, and 61. This was most evident in codon 12 for GTT and TGT mutations, codon 13 for CGC, and codon 61 for CTA mutation.

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Table 3 (*continued*)

Citation	Comparison Endpoint	Agent	Animal Model	Tumor	Evaluation/Comment
Sills et al. (1999a)	Oncogene comparison	Chloroprene	B6C3F1	Lung	While a significant mutation spectra difference occurred within the control and treated groups/tumors for oncogene mutations, it was most evident for codon 61.
Sills et al. (2001)	Oncogene comparison	1,3-butadiene; isoprene; chloroprene	B6C3F1	Forestomach	The exposure to these agents induced changes in ras mutations which were a qualitatively and quantitatively different mutation spectrum than in spontaneously occurring neoplasms of the forestomach. The lack of H-ras codon 61 CTA mutations and the rare detection of K-ras codon 13 CGC mutations in the spontaneous forestomach neoplasm enhances the likelihood for a causal link with the chemical exposure.
Stanley et al. (1992)	Oncogene comparison	Vinyl carbamate	C57BL/6 J B6D2F1	Liver	These strains of mice are somewhat resistant to developing chemically-induced liver tumors and the frequency spectra of mutations for codon 61 was different in induced and spontaneous tumors, suggesting that different oncogene pathways were activated.
Ton et al. (2004)	Oncogene comparison	BMP 2,2-bis(bromomethyl1,3-propanediol) TNM	B6C3F1 mice	Lung tumors	The treated mice had lung tumors with K-ras mutations, most being GGT- > GTT mutations in codon 12, while none were observed in controls. The lung tumors typically show G- > A transitions (GGT- > GAT) at codon 12 (13/18), while none occurred with spontaneous lung tumors.
Unfried et al. (2002)	Oncogene comparison	Asbestos: crocidolite	(transgenic) F344 female rats, 8 weeks	Greater omentum mutations spectra lacl gene from DNA of omenta of lacl transgenic rats	The molecular assessment of the mutations showed marked differences (mutation types) between asbestos-induced mutations and spontaneous mutations. The authors concluded that a specific molecular mechanism induced by crocidolite differs from that induced by the generation of spontaneous mutations. G to A transitions occurred in the spontaneous mutations with a frequency of 57%. This occurred with only 20% frequency with crocidolite and 35% for BaP. Chromosome deletions were also 5 times more common with crocidolite than in spontaneous mutations.
Warshawsky et al. (1996)	Oncogene comparison	7H-dibenzo[c,g]carbazole	A/J	Lung tumor	K-ras mutations were very different for treated mice at codon 61 than spontaneous tumors. Multiple mutations at codon 12 for spontaneous tumors did not occur for treated mice.
Watson et al. (1995)	Oncogene comparison	Vinyl carbamate	B6C3F1	Hepatocellular tumor	Differences in mutation spectra for codon 61 are shown with CTA adenomas.
Wiseman et al. (1986)	Oncogene comparison	N-HO-AAF; VC (vinyl carbamate) HO-DHE (1′-hydroxy-2′,3′-dehydroestragole)	B6CeF1 male mice	Hepatoma	If the mutations included by these three agents occurred from spontaneous mutagen events, the distribution of mutations should have been similar regardless of the specific chemical treatment (see Discussion), which was not the case
You et al. (1989)	Oncogene comparison	BaP; MNU EC-ethylcarbamate	Strain A	Lung tumors	Spontaneous and chemically-induced lung tumors displayed a significant “different mutation spectra seen with codon 12 – TGT for BaP, codon 12 for GAT for MNU, and codon 61 for CTA for EC.
You et al. (1993)	Oncogene comparison	Benzotrichloride (BTC)	A/J mice	Lung tumors	The pattern of activating mutations in the K-ras gene seen of BTC-induced lung tumors differed markedly from the spontaneously occurring lung tumors. Activating mutations in spontaneous tumors were in both codon 12 (60%) and codon 61 (30%) with several types of codon substitute transitions. In contrast, 100% of the activating mutations detected in BTC induced lung tumors were in the 12th codon with exclusively GC- > AT transitions. Only 27% of the K-ras mutations seen in spontaneous tumors were GC- > AT transitions. These observations revealed that the activating mutations in the K-ras gene of BTC-induced lung tumors probably were induced by a genotoxic effect of the agent.

spontaneous and induced tumor biology occurred as an outgrowth of the discovery of oncogenes at the level of molecular pathology (Tables 3 and 4). This research involved both the development of basic understanding of oncogenes in the process of carcinogenesis and using these findings to assess the predictive utility of the NTP cancer bioassay program (Maronpot et al., 1995).

Over the course of the now three decades, this area of research has expanded to include 45 agents (Table 5), multiple animal models (Table 6) and the assessment of multiple tumor types (Table 7). Furthermore, the mechanistic evaluation has progressed beyond initial oncogene activation to the occurrence of patterns of gene activation/molecular pathways (Blackshear et al., 2015), and the subsequent occurrence of additional mutations (Parsons et al., 2010) which further transform the altered tissue towards a more tumor-like progression, linked to metabolic reprogramming, creating progressively modified tumor phenotypes (Martinez-Outschoom et al., 2013; Kerr et al., 2016). This mechanistic based knowledge indicated that tumors become progressively more diverse with individualistically appearing phenotypes, enhancing the difficulty for successful therapeutic applications (Salk et al., 2010). This general progress of knowledge of tumor development revealed that the induced tumors can be molecularly differentiated from similar appearing spontaneous tumors (Tables 3 and 4). This general biological/molecular framework does not support the assumption that one could expect additive to background for cancer assessment since spontaneous and induced tumors that develop within the same organ/tissue typically do not display the same mechanism(s).

The capacity to make molecular mechanism evaluations of the additive to background assumption emerged during the mid-1980s with the identification of proto-oncogenes and their related molecular pathways, as well as methodological developments such as polymerase chain reaction (PCR) in the late 1980s and other nucleotide assessment methods. None of the numerous subsequent experimental papers published on the issue of whether induced tumors employed the same mechanisms of background/spontaneous tumors using mutated/activated oncogenes related their findings back to the issue of cancer risk assessment, regulatory agency policy, or the additive to background papers of Amitage and Doll (1957), Crump et al. (1976), Guess et al. (1977), or to the guidance of the NAS SDWC (1977) or the U.S. EPA (1986, 1996, 2005) cancer risk assessment guidelines which reaffirmed the additive to background assumption.

3.5. Direct comparison: Spontaneous vs Induced Tumor Mechanisms

Data that supported the hypothesis that spontaneous and induced tumors were not mediated by the same mechanistic process emerged during the late 1980s to mid-1990s. A substantial number of the studies as summarized in Tables 8–18, and Figs. 3–10 compare spontaneous and induced tumors for oncogene mutation. When the carcinogen treatment induces different mutations and/or different mutation patterns than those of the spontaneous tumors it suggests that the carcinogen induced tumor occurred via different mechanism(s) than those involved in spontaneous tumors (Maronpot et al., 1995-page 132). An example of such a carcinogen induced altered mutation spectra is seen in Fig. 3, which compares spontaneous and NDEA-induced liver H-ras oncogene mutations in the B6C3F1 mouse with respect to codon 61 induced mutations at the three positions. The figure indicates that 64.6% of the spontaneous hepatocarcinomas display mutations in H-ras codon 61 whereas in the NDEA-induced tumors 46.7% have H-ras oncogene mutations (Chen et al., 1993). The codon 61 mutations of the treated mice were distributed in a manner that differed markedly from spontaneous tumors. None of the treatment induced tumor mutations were in position #1 (AAA) of codon 61 while position #2 (CGA) displayed nearly 93% of the mutated oncogenes. These data indicate that the induced tumors displayed significantly less mutated ras oncogenes at position #1 and a profoundly different overall mutation spectra pattern. These findings suggest that the tumor mechanisms are significantly different between the spontaneous and induced tumors. The findings of Nesnow et al. (1995) describe responses with the A/J mice strain lung tumor model for four polycyclic aromatic hydrocarbon carcinogens for codon 12 (Table 16). As was the case with the B6C3F1 mouse liver, these findings also illustrate marked differential patterns of oncogene involvement in tumor development between the chemically induced and spontaneous tumors. Tables 8–18 and Figs. 3–10 include a wide range of chemical agents with a predominant focus on hepatocellular carcinoma in the B6C3F1 mouse for K-ras mutations. However, the listing is also broadly inclusive of other animal models, tumor types, and oncogenes. While each mutation spectra is specific to the agent, model, and endpoint, the overall pattern of marked differences between induced and spontaneous tumor appears independent of the diverse experiment parameters (e.g., agent, model, tumor, etc.).

An assessment of numerous similar types of studies reveal marked carcinogen-specific oncogene mutation frequencies and spectra. Tables 8–18 and Figs. 3–10 show such cases of significantly differing mutation spectra for a wide range of chemical agents for induced versus spontaneous tumors. These findings indicate that the induced tumors are very unlikely to act via the identical mechanisms of the spontaneous tumors that relate to the specific oncogenes studied (Table 19). The tables reveal that the additive to background assumption was not credible for 45 agents, many of considerable environmental public health relevance (Table 5). These studies affected a broad range of animal models (Table 6) and tumor types (Table 7).

In a dose-time response extension of the above oncogene research, McKinzie and Parsons (2011) assessed oncogene-related mutation frequency over 32 weeks, with a temporal linkage of oncogene activation and colon cancer in the F344 rats treated with azoxymethane (AOM). The GAT and GTT mutations of codon 12 were not altered over the first 24 weeks in controls. However, by week 32, the control group displayed an increase in codon 12 mutations. The control developmental oncogene mutation pattern sharply differed from the AOM-treated rats, which displayed a significantly enhanced mutation rate of codon 12 after only one week of exposure. According to McKinzie and Parsons (2011), the findings reveal that the occurrence of cancer due to age in the control rats displays a different mechanism and progression pattern for the same type of tumor that results from the AOM treatment.

The concern expressed by Hoel (1997) about the compatibility of additivity to background assumption with advances in molecular biology may be illustrated further in the paper of Hisamoto et al. (2007), which compared oncogene mutations in cisplatin, NNK and BaP induced lung tumors of the A/J mouse. In the cisplatin-induced tumors there was an absence of K-ras codon 12 mutations, which is known to be the key mutations-induced by NNK or BaP for lung tumor development. The authors concluded that cisplatin induced the same type of lung tumor in the A/J mice as the NNK or BaP, but by a different mechanism. The same type of tumor in the same animal model may, therefore be produced by different mechanisms confirming the issue raised by Hoel (1997) that molecular biology advances may provide the means to assess the validity of the additive to background assumption.

3.6. Carcinogen-mediated background response reprogramming

Metabolic reprogramming by activated/mutated oncogenes has assumed an important role in tumorigenesis (Martinez-Outschoom et al., 2013; Kerr et al., 2016). The process from oncogene activation to metabolic transformation often leads to glycolytic reprogramming of tumor cells. The rewiring is a progressive process, with the subsequent occurrence of newly activated and deactivated oncogenes, which mediate the creation of novel tumor phenotypes as well as the regression of tumorigenetic processes. Many of the studies in the present article that compare spontaneous and induced tumors reveal that carcinogen treated animals show a transformed mutation spectra. The carcinogen treatment not only induces a specific-mutation spectra signature, but often significant changes in a highly predictable/expected (i.e., background)

Table 4
How decisions can be made concerning whether additive to background tumor risk can be assumed. Spontaneous vs chemically/radiation-induced tumor comparison.

Comparison Area #1	Comparison Area #2	Comparison Area #3	Comparison Area #4	Comparison Area #5
Origin/location of tumor in affected organ/tissue Example: Chinsky et al. (1985)	Global gene expression; profile of tumors Examples: Blackshear et al., (2014, 2015); Imaoka et al. (2008); Marxfeld et al. (2006);	Mutation profile of tumor based on codon comparison with oncogenes, often with Ha-Ras, K-Ras and N-Ras Examples: Codons 12, 13, 61; Manam et al., (1992, 1995); Rumsby et al. (1991); Fox et al. (1990); You et al., (1989, 1993); Sills et al., (1995, 2001); Donnelly et al. (1996); Mass et al. (1996); Hayashi et al. (2001); Ton et al. (2004); Hong et al. (2015); Watson et al. (1995); Stanley et al., 1992	Mutation spectra comparison in target tissue for spontaneous and treatment induced mutations Examples: Mittelstaedt et al. (1999); Unfried et al. (2002); Hong et al. (2003); Reynolds et al., (1986, 1987, 1988); Nishimura et al. (1999); Iizuka et al. (2010); Kawano et al. (1996); Chen et al. (1993)	Loss of heterozygenicity Example: Antal et al. (2002)

General Comparison Framework

- Identify Key Proto-oncogenes thought to be involved in the process of organ-specific spontaneous and chemically/radiation induced similar tumors (e.g., lung, liver).
- Compare proportion of tumors with specific oncogenes and oncogene subtypes. For example, RAS oncogenes and its subtype H-ras, K-ras, and N-ras.
- Assess/compare changes within specific sub oncogenes for changes within specific codons: For example, ras oncogene – codon 12, 13, and 61.

Five Comparison Areas: Anyone may be employed to assess additive to background within a specific study.

oncogenic activation spectra (Nesnow et al., 1995). Anna et al. (1994) refer to such carcinogen-induced changes as “selective inhibition and enhancements of subsets of spontaneously initiated hepatocellular neoplasms”. Tables 16–18 provide examples of multiple carcinogen treatments that affect significant changes in oncogene activation of spontaneous tumors. These findings not only contradict the assumption of additivity to background, but also challenge the traditional concept of background after carcinogen treatment and whether the control group can serve as a legitimate reference group.

The carcinogen treatment, therefore, does not necessarily only add to the oncogene mutation spectra, but has the potential to fundamentally reprogram and transform it. The long-held assumption that the carcinogen treatment directly adds to identical spontaneous mutation frequency and spectra is not supported by a substantial number of studies assessed here. While spontaneous tumor oncogenic transformation/reprogramming occurs, specific spontaneous and induced tumor studies have only been assessed, to a limited extent, developmentally and/or for time course changes of this striking transformation (McKinzie and Parsons, 2011). Nevertheless, such reprogramming transformations may be generalized across biological model, organ, and inducing agent (Table 16).

While carcinogen exposures significantly alter the frequency of K- and H-ras oncogene mutations and tumor mutation spectra, there has been limited research with other oncogenes or other biological processes. Jackson et al. (2006) have produced a type of oncogene mutation meta-analysis for induced vs spontaneous tumors, for mouse liver H-ras and human liver TP53 mutations. This integrative summary of human studies re-enforces the above findings, illustrating fundamentally different general patterns of oncogene mutation responses between induced and spontaneous tumors. These findings strongly challenge the concept of additive to background. It is, however, also evident that the administration of the carcinogen treatment can alter the normal course of spontaneous oncogene activation, while imposing its own unique agent induced mutation spectra. These findings demonstrate that the carcinogen treatment in these instances does not only add to but may significantly modify the background response.

3.7. Dose-dependent oncogenic mutations

Significantly different mutation spectra may be induced by the same carcinogen in a dose dependent manner (Sills et al., 1999a; Hong et al., 2015; Cazorla et al., 1998; Zhuang et al., 2002; Manam et al., 1995; Nishimura et al., 1999; Warshawsky et al., 1996; Hecht et al., 1998; Chen et al., 1993). These findings indicate that different tumor-inducing mechanisms can operate at different doses. Both Bogen (2017) and Crump (2017) have acknowledged the occurrence of high and low dose slope divergence and its significant implications for modeling low dose effects. The presence of different mechanisms of oncogene activation across dose further challenges the process of decision making with respect to dose-response modeling which depends on the additive to background assumption (Blackshear et al., 2014, 2015; Imaoka et al., 2008). These findings show not only the evolving complexities of mechanistic understandings of induced vs spontaneous tumors, they also highlight the limitations of the initial additive to background assumption.

4. Discussion

The decision to adopt additive to background by the U.S. EPA for cancer risk assessment was influenced by the U.S. NAS SDWC (NAS, 1977) based on the Crump et al. (1976) paper, as well as the OSHA (1980) Carcinogen Hearings and Hoel (1980). Schneiderman, a member of that SDWC, summarized its recommendations in a follow-up paper, indicating that known carcinogens have never induced cancers in people that have never been seen before (i.e., novel cancers) (Schneiderman and Brown, 1978). With this logic, it was assumed that

Table 5
Agents used to assess additive to background hypothesis for induced cancer.

	Agents
1	1,3-butadiene
2	2,2-bis(brom-methyl-1 to 3 propanediol
3	2AAF
4	4-amino azobenzene
5	5-methylchrysene
6	ADBA
7	Aflatoxin B
8	Asbestos crocidolite
9	Azoxymethane
10	Benzene
11	Benzo(a)pyrene
12	Benzo(b)fluoranthene
13	Benzidine
14	Benzotrichloride
15	Chloroform
16	Chloroprene
17	Ciprofibrate
18	Cobalt Metal
19	Cumene
20	Cyclopental(cd)pyrene
21	DEN
22	DMBA
23	Ethyl carbamate
24	Foran
25	Furfural
26	gamma-radiation
27	HO-DHE
28	Isoprene
29	Methylene chloride
30	MCA
31	MNU
32	N-HO-AAF
33	NDEA
34	NDMA
35	NNNK
36	O-nitrotoluene
37	Ozone
38	Phenobarbitone
39	Radionuclides
40	Riddelliine
41	TCDD
42	Tetranitromethane
43	Vanadium pentoxide
44	Vinyl carbamate
45	Vinylidene chloride

Table 6
Animal model utilized to assess additive to background hypothesis.

Animal Models
A/J mice
AC3F1 (A/J x C3H/He5)
B6C3F1 mice
C3H mice
C3H/Hp mice
C57BL/6 × C3H/He mice
C57BL/6 × DBA2 mice
CD-1 mice
RF/J/AKR mice
Scid mice
Sprague-Dawley rats
Strain A mice
F344 rats

carcinogens will act at least to some extent via an identical mechanism (s) in the same target cells as occurs with normal background conditions. This perspective lead Schneiderman and Brown (1978) (and the NAS SDWC) to conclude that exogenous carcinogens act in an additive to background manner within humans. Populations already exposed to endogenous and background environmental carcinogens were, therefore, expected to experience cancer risks in a continuous (i.e., linear-progressive-non-stepwise) manner with respect to dose, regardless of how low the dose. In their paper, Schneiderman and Brown (1978) acknowledged the assistance of several key individuals such as David Rall (the NIEHS Director), David Hoel (NIEHS biostatistician) and Sheldon Murphy (former President of the U.S. Society of Toxicology), all members of the NAS SDWC for the writing of his article, each of whom offered comments at the OSHA (1980) hearings except Murphy.

The above NAS SDWC recommendation and explanation of Schneiderman and Brown (1978) were subsequently challenged and largely contradicted by a substantial body of literature from the late 1980s to the present, showing that experimentally induced tumors display significantly different types of oncogene mutations and profoundly differing oncogene mutation spectra than spontaneous tumors (Figs. 3–10; Tables 8–18). The early and seemingly reasonable belief that when a chemical/ionizing radiation induces the same type of tumor in the same organ (e.g., lung, liver, etc) as does the control group, it does so via the same mechanism is not the case. In fact, these studies reveal no convincing evidence that induced and spontaneous tumors are even infrequently likely to be mediated by the same mechanisms. This conclusion is supported by observations that as the process of carcinogenesis progresses, more tumor specific changes occur, resulting in unique tumor specific phenotypes (see subsection entitled: Tumor Mutation Complexities) (Salk et al., 2010; Kerr et al., 2016). Further challenging the original additive to background decision is the growing view that most tumors are heterogeneous, suggesting that multiple clones of complementary mutant cells may influence the tumor progression (e.g., colon tumor) (Parsons, 2008; McKinzie and Parsons, 2011; Vogelstein et al., 2013).

The original additive to background assumption for cancer was based upon a mutation mechanism. It did not address the question of epigenetic carcinogens. Yet, the EPA procedure has made no distinction between genotoxic and epigenetic carcinogens with respect to the additive to background assumption. In 1994, Ray et al. (1994) reported that phenobarbital (PB)-induced liver tumors in a mouse model via epigenetic mechanisms. The molecular mechanisms that mediated the PB induced liver tumors were different than those of spontaneous tumors. This mechanistic divergence between epigenetic carcinogens and mechanisms and time of spontaneous tumors have been reported in other experimental studies (Hegi et al., 1993). Such findings suggest that the additive to background assumption may also not be applicable for chemically induced epigenetic tumors as well.

The limitations of the additive to background concept for environmental induced cancer was suggested by the results of a substantial series of ionizing radiation induced gene mutation studies. As has been the case with additive to background for chemically induced cancer, this same hypothesis for ionizing radiation induced mutations, as researched in considerable depth by Stadler, Muller, and others from the 1930s to 1950s, was not supported (Roman, 1988; Stadler, 1954; Stadler and Roman, 1948). Had the chemical toxicology and risk assessment communities of the 1970s and early 1980s better appreciated the history of the spontaneous and ionizing radiation induced mutation historical literature, the additive to background assumption may not have been incorporated into regulations by U.S. EPA (1986), reflecting the historical truism of the Spanish philosopher and novelist, George Santayana (1863–1952) that “who cannot remember the past is condemned to repeat it.”

4.1. Reprogramming the disease background

The same tumor types/in the same organ of the same biological model for spontaneous and induced tumors typically show significant differences in oncogene activation and mutation spectra. Furthermore, the treatment with chemical carcinogens may significantly alter the occurrence of oncogene activation/mutation as would occur in

Table 7
Tumor types utilized to assess additive to background hypothesis.

Tumor Types
Colon
Forestomach
Harderian gland
Liver
Lung
Lymphoma
Mammary
Mesothelioma
Thymic

spontaneous tumors, revealing a type of developmental cellular/tissue rewiring/reprogramming. The fundamental assumption of additive to background for carcinogens is, therefore, not compatible with the mutation spectra data. These developments are also incompatible with the concept of independence of background since the carcinogen treatment reprograms the normal spontaneous tumor response by changing the background of the animals allocated to the treatment groups. This observation challenges the traditional concept of a control group and how comparisons to a control group are to be made.

The carcinogen-induced reprogramming process may offer an insight to the findings of Gray et al. (2002) that many chronic bioassays display striking mixtures of organ-specific tumor and antitumor responses relative to the control. While the standard comparison to the control is valid in these cases, the issue of additive and independent of background will need to be re-evaluated within the context of the chronic bioassay related cancer dose-response assessment. The issue of carcinogen-induced reprogramming presents new challenges and opportunities that may affect model and dose selection, mechanism evaluation, carcinogen risk assessment modeling, and risk estimates currently used that affect numerous societal areas including health, medical, legal, and technology domains.

Table 8
Pattern of K-ras mutations in chloroprene and isoprene in lung neoplasms from female B6C3F1 mice (Source: Sills et al., 1999, Table 1, Page 659).

	Codon 12 (GGT)						Codon 61 (CAA)			
	G**T**T	G**A**T	T**G**T	C**G**T	C**T**T	A**T**T	c**T**A	c**A**T	c**A**C	c**G**A
Spontaneous	6.6	60.0	33.3	0	0	0	0.0	57.1	14.2	28.4
Chloroprene	20.0	50.0	0	10.0	10.0	10.0	88.0	0	0	12.0
Isoprene	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0

Note: Numbers in table represent percent of specific oncogene mutations within a specific codon. For example, the TGT mutation represents 33.3% of the total oncogene mutations for codon 12. The total mutations sums to 100% within each codon for spontaneous and agent induced tumors. Bolded, enlarged letters represent the location of mutation in codon for Table 8–Table 18.

Table 9
K-ras mutations in Harderian gland neoplasms and lung tumors for ethylene oxide-exposed B6C3F1 mice. (source: Hong et al., 2007).

Harderian Gland Tumors							
	Codon 12 (GGT)		Codon 13 (GGC)		Codon 61 (CAA)		
	G**A**T	T**G**T	C**G**C	A**G**C	c**T**A	c**G**A	c**A**G
Spontaneous	0.0	0.0	0.0	0.0	100	0.0	0.0
Ethylene Oxide	3.3	26.6	50.0	3.3	3.3	10.0	3.3
Lung Tumors							
	Codon 12 (GGT)			Codon 13 (GGC)		Codon 61 (CAA)		
	G**A**T	T**G**T	G**T**T	C**G**T	A**G**C	C**G**C	c**G**A	c**A**T	c**A**C
Spontaneous	40.7	18.5	3.7	0.0	0.0	1.11	7.4	14.8	3.7
Ethylene Oxide	9	0	84	0	4	0	4	0	0

Note: Numbers in table represent percent of specific oncogene mutations within codons 12, 13, and 61. For example, the TGT mutation in codon 12 for Harderian gland tumors represents 26.6% of the total oncogene mutations for codon 12, 13, and 61 summed together. The total mutations sums to 100% across the three codons for spontaneous and agent induced tumors for Harderian gland and lung tumors.

Table 10
Summary of K-ras mutations in alveolar/bronchiolar tumors in B6C3F1 mice from various NTP bioassays (source: Hong et al., 2015, Table 8, page 878).

	Codon 12 (GGT)				
	GAT	TGT	GTT	CGT	N
Spontaneous (Historical Control)	70	25	5	0.0	20
Cobalt metal dust[a,b]	16.6	3.3	76.6	3.3	30
1,3 butadiene[b]	Insignificant response	Insignificant response	Insignificant response	Insignificant response	Insignificant response
Ethylene oxide[a,b]	8.6	0.0	91.3	0.0	23
Cumene[b]	25.0	20.8	45.8	12.5	24
2,2-Bis(bromomethyl)-1,3-propanediol[c]	71.4	3.6	25.0	0.0	28
Tetranitromethane[a,b]	100.0	0.0	0.0	0.0	13
Isoprene[b]	0.0	0.0	0.0	0.0	0.0
Methylene Chloride[b]	-	-	-	-	-
	Codon 61 (CAA)					
	CAC	CTA	CGA	CAT	Other[d]	N
Spontaneous (Historical Control)	0	0	37.5	50.0	12.5	12
Cobalt metal dust[a,b]	0	0	35.7	28.5	35.7	14
1,3 butadiene[b]	0	66.6	33.3	0	0	9
Ethylene oxide[a,b]	Insignificant response	Insignificant response	Insignificant response	Insignificant response	Insignificant response	Insignificant response
Cumene[b]	Insignificant response	Insignificant response	Insignificant response	Insignificant response	Insignificant response	Insignificant response
2,2-Bis(bromomethyl)-1,3-propanediol[c]	Insignificant response	Insignificant response	Insignificant response	Insignificant response	Insignificant response	Insignificant response
Tetranitromethane[a,b]	0	88.0	12.0	0.0	0.0	25
Isoprene[b]		0.0	100.0	0.0	0.0	10
Methylene Chloride[b]	57.1	14.2	14.2	14.2	0.0	7

Note: Numbers in table represent percent of specific oncogene mutations within a specific codon. For example, the spontaneous TGT mutation represents 25% of the total oncogene mutations for codon 12. The total mutation percentages sum to 100% within each codon for spontaneous and agent induced tumors.
[a] If the same tumor had 2 point mutations, it was counted as 1.
[b] Exposure by inhalation for ~ 2 years.
[c] Exposure by feed for ~ 2 years.
[d] Corrected from original reference.

4.2. Pre- post-conditioning and "subtraction" from background

While the above assessment indicates that chemically and radiation-induced tumors typically act via mechanisms that differ from those of the same type of tumors in control groups (i.e., spontaneous tumors), other experimental evidence further weakens the additive to background assumption. These include the concepts of pre- and post-conditioning/adaptive response (Calabrese, 2016a, 2016b) and the lack of induced genetic changes at ionizing radiation doses far greater than background (Russell, 1969; Olipitz et al., 2012). In the case of preconditioning, a prior low dose of a vast array of toxic chemicals, radiation and other stressor conditions may reduce damage from a subsequent and more substantial toxic exposure. The decreased damage under optimized conditions is typically in the 30–60% range. The shape of the preconditioning/adaptive response follows an hormetic dose response (Calabrese, 2016a, 2016b). The preconditioning/adaptive response reflects a biological "subtraction" phenomenon, in which the subsequent exposure to mutagens and other toxic agents can be significantly less than additive, discrediting the additive to background assumption within such experimental settings. The preconditioning/adaptive response typically has two functional windows of protection over a several day period (i.e., short-term – 1 h. and then long term starting again at 12 h. and continuing for about 48–72 h). There is also a limited effective dose that typically involves a 25–200 fold range (Calabrese, 2016a, 2016b). While the preconditioning/adaptive response is highly generalizable, the specific experimental parameters may vary by dose, model, endpoint and agent. Furthermore, not only may damage be significantly mitigated by a prior low dose of numerous

Table 11
K-ras mutations in lung neoplasms of B6C3F1 mice in a two-year inhalation study of cumene (source: Hong et al., 2008, Table 2, page 722.

	Cumene								
	Codon 12 (GGT)			Codon 13 (GGC)		Codon 61 (CAA)			
	GAT	TGA	GTT	CGT	CGC	CGA	CAT	CAC	CTA
Spontaneous (Historical Controls)	42.4	15.1	3.0	0	18.2	6.1	12.1	3.0	0
Cumene Treated Groups	13.3	11.1	24.4	6.6	8.8	28.2	0	4.4	2.2

Note: Numbers in table represent percent of specific oncogene mutations acrosscodons 12, 13, and 61. For example, the TGA mutation represents in codon 12 represents 15.1% of the total oncogene mutations for codons 12, 13, and 61 summed together. The total mutations sums to 100% across the three codons for spontaneous and agent induced tumors.

Table 12
H-ras mutation spectrum in benzene-induced B6C3F1 mouse mammary carcinomas (source: Houle et al., 2006, Fig. 4, page 759).

Benzene			
	Codon 61 - CAA		
	A to G	A to T	C to A
Spontaneous Control	71.0	14.3	14.3
Benzene	40.0	40.0	20.0

Note: Numbers in table represent percent of specific oncogene mutations within codon 61. For example, the A to G mutation in the spontaneous tumors represents 71% of the total oncogene mutations for codon 12. The total mutation percentages sum to 100% within codon 61 for spontaneous and agent induced tumors, respectively.

Table 13
p53 mutation base preference in benzene-induced B6C3F1 mouse mammary carcinomas (source: Houle et al., 2006, Fig. 5, page 759).

p53				
	Codons 5–8			
	G	A	T	C
Spontaneous Control	8	24	0	68
Benzene	44	28	14	14

Note: Numbers in table represent percent of specific oncogene mutations across codons 5–8 for the p53 gene. For example, the G mutation for the benzene treatment represents 44% of the total oncogene mutations for codons 5–8 summed together. The total mutations sums to 100% across codons 5–8 for spontaneous and agent induced tumors, respectively.

Table 14
H-ras mutation spectrum in ethylene oxide-induced B6C3F1 mouse mammary carcinomas (source: Houle et al., 2006, Fig. 4, page 759).

	Codon 61 (CAA)		
	A to G	A to T	C to A
Spontaneous Control	0.0	20.0	80.0
Ethylene Oxide	40.0	40.0	20.0

Note: Numbers in table represent percent of specific oncogene mutations within codon 61. For example, the A to G mutation in the ethylene oxide tumors represents 40% of the total oncogene mutations for codon 61. The total mutation percentages sum to 100% within codon 61 for spontaneous and agent induced tumors, respectively.

Table 15
p53 mutation base preference in ethylene oxide-induced B6C3F1 mouse mammary carcinomas (source: Houle et al., 2006, Fig. 5, page 759).

p53				
	Codons 5–8			
	G	A	T	C
Spontaneous Control	10.0	20.0	0.0	70.0
Ethylene Oxide	60.0	10.0	10.0	20.0

Note: Numbers in table represent percent of specific oncogene mutations across codons 5–8 for the p53 gene. For example, the G mutation for the ethylene oxide treatment represents 60% of the total oncogene mutations for codons 5–8 summed together. The total mutations sums to 100% across codons 5–8 for spontaneous and agent induced tumors, respectively.

toxic agents/carcinogen agents, a similar extensively documented phenomena (i.e., post-conditioning) can also diminish harmful effects when given after the toxic exposure within a limited time window. These significant scientific developments emerged one to two decades after the Crump et al. (1976) paper.

The conditioning treatment may act not only to reduce damage to a subsequent challenging dose but also to reduce damage from ongoing prior disease processes, creating a type of subtraction from background phenomenon (Calabrese, 2018). A post-conditioning type of subtraction from background was reported by Azzam et al. (1996). They found that a very low total dose of (0.1 cGy; 0.24 cGy/min) [i.e., equivalent to about one year of background (non-radon) gamma ray radiation delivered in less than one minute] upregulated repair mechanisms in C3H 1OTV2 cells, reducing neoplastic transformation by approximately 60–80%. The test dose of 0.1 cGy was selected to ensure that each cell that was hit received (on average) about one track. According to the Azzam et al. (1996) the exposure selected reflected a real-life increase of dose between zero (including background) and the dose deposited by one track (i.e., background does not yield one track for each cell on average). The single track dose in this cell system not only did not increase the frequency of neoplastic transformation but significantly reduced it over the range from 0.1 to 10 cGy. The findings of Azzam et al. (1996) were strongly supported by a detailed mechanistic

Table 16
Differential pattern of oncogene involvement in PAH-induced lung cancer in the A/J mouse for spontaneous vs induced tumors for four carcinogens (source: Nesnow et al., 1995).

Codon 12 (GGT)								
Spontaneous Lung Tumor[1]				Induced Lung Tumor				
TGT	GTT	GAT	CGT		TGT	GTT	GAT	CGT
0.0	33	59	8	BaP[a]	57	24	19	0.0
0.0	33	59	8	B(b)F[b]	61	39	0.0	0.0
0.0	33	59	8	5MC[c]	50	22	0.0	28
0.0	33	59	8	CPP[d]	25	15	10	50

[1] Numerical values represent percent (%) of total mutations (100%) for the spontaneous tumors and agent-induced mutations of codon 12.
[a] BaP = benzo(a)pyrene.
[b] B(b)F = benzo(b)fluoranthene.
[c] 5MC = 5-methylchrysene.
[d] CPP = cyclopental[cd]pyrene.

Table 17
Pattern of H-ras gene mutations at codon 61 in liver tumors from male B6C3F1 mice treated with genotoxic chemicals (Source: Anderson et al., 1992).

		Codon 61 (CAA)		
Chemical	Tumors with Activated H-ras (%)	AAA	CGA	CTA
Spontaneous	61/91 (67%)	37 (62.6%)	16 (26.2%)	8 (13.1%)
Vinyl carbamate (VC)	29/37 (78%)	2 (5.4%)	1 (2.7%)	26 (89.6%)
dimethylbenz(a)anthracene (DMBA)	10/10 (100%)	0 (0.0%)	0 (0.0%)	10 (100%)
N-hydroxy-2-acetylaminofluorene (N-OH-AAF)	7/7 (100%	7 (100%)	0 (0.0%)	0 (0.0%)
benzidine (BZD)	13/22 (59%)	11 (84.6%)	1 (7.7%)	1 (7.7%)
N-nitrosodiethylamine (DEN)	14/33 (42%)	7 (50%)	3 (21.4%)	4 (28.6%)

Numerical values represent percent (%) of total mutations (100%) for the spontaneous tumors and agent-induced mutations of codon 61.

radiobiological model (Schollnberger et al., 2002), experimentally confirmed and significantly extended by others to lower doses (Redpath et al., 2001, 2003a, 2003b; Redpath and Elmore, 2007; Ko et al., 2004). These findings indicate that over a relatively broad dose range above background post-conditioning subtraction from background risk may occur.

The pre- post-conditioning/adaptive response phenomena are very general and have application to the additive to background assumption for both carcinogens and non-cancer endpoints.

4.3. Threshold responses and the additive to background concept

Within the context of additive to background, a continuous exposure to 0.0002 cGy/h., a dosage approximately 400 fold greater than background, for five weeks in a mouse model did not induce detectable changes in DNA nucleobase damage products. Likewise, neither was there evidence of DNA fragmentation in the micronucleus assay assuming double strand breaks. Further, at this dose rate a wide range of gene transcripts of DNA damage responses were not induced. Of significance is a dose-rate of about 30 fold higher than background exceeds permissible human exposures (FEMA, 2002). Olipitz et al. (2012) failed to show a response that is additive to background at a dosage some 400 fold greater than background even with very sensitive biomarkers of genetic damage. Similar findings were reported over a 400 day exposure period of 1 mGy/22 h day or 400 fold higher than background in a mouse model (Ono, 2013; Tatsumi and Tanooka, 2014). In addition, the massive data of Russell (1969) with the mouse specific locus test failed to induce mutations in oocytes at doses up to 27,000 fold greater than background. While these data of Russell (1969) were known to the NAS within the BEIR I Genetics Subcommittee (NAS/NRC, 1972) and the Safe Drinking Water Committee (1977) they had no discernable impact on their risk assessment recommendations.

4.4. The problem of background exposure and the dose-response relationship in the area of zero dose

In the case of ionizing radiation, it has long been assumed that one cannot ignore the occurrence of background exposure. Why? From before birth the individual has exceeded zero dose. By the time this

Table 18
H-ras activation at codon 61 in hepatocellular adenomas and carcinomas following treatment with VC and/or TCDD (sexes combined) (source: Watson et al., 1995, page 1707).

Spontaneous Tumors				Induced Tumors		
Codon 61 - CAA				Codon 61 - CAA		
AAA	CGA	CTA		AAA	CGA	CTA
66.6	33.3	0.0	Vinyl Carbamate (VC)	18.5	18.5	63.0
			TCDD	69.5	17.5	12.5
			VC + TCDD	11.3	15.1	73.6

Numerical values represent percent (%) of total mutations (100%) for the spontaneous tumors and agent-induced mutations of codon 61.

person is 35–50 years of age background dose may be expected to be 70–100 mSy. According to the traditional radiation geneticist mantra, the body only recognizes the total dose received. Now if the person received an additional dose (i.e. see Beninson, 1988 - Fig. 5, Page 447) the body is assumed to simply add this to the background, proportionately enhancing risk. **"It is this incremental dose risk proportionality that is normally referred to as the linear non-threshold hypothesis"** (Beninson, 1988). The risk assessment "management system" for occupationally exposed people needs to deal with two type of exposures in such a manner that the summated risk is the same whether exposure A occurs first followed by exposure B or the other way around. For the risk management system to work the risk must be additive. If this were not the case, one could not add up the various dose increments that occur throughout the exposure period (e.g. a working week or year). It is the accepted convention of adding up the incremental doses that are received throughout the given period. This process creates the capacity to standardize and control the annual doses received by workers. This is the key management equity argument that legitimizes equal treatment across workers. This regulates the dose distribution (i.e. dose rate) within the work period (e.g. 1 year) (see Beninson, 1988 – Fig. 6, page 447). The risk associated with the total permitted dose is assumed within the given time period. According to Beninson (1988) "This is the basic condition for a workable dose control system in radiation protection".

This statement of Beninson (1988), who was chair of the International Commission of Radiological Protection (ICRP) at the time of the statement, is of relevance to the additive to background assessment. Beninson based his belief in linear cancer risks on the assumption that mutations also show a linear dose response down to a single ionization. He noted that these effects are contingent upon the transformation of a single cell and would therefore conform to a probabilistic/stochastic framework rather than a non-stochastic effect. He then indicated that the probability of inducing transformed cells will be equal to one minus an exponential function of dose, with the responses at low doses being "approximately linear." He later cited micro-dosimetric arguments indicating that the dose response should be linear even when there is even less than one energy track on average per cell.

With this as his theoretical background argument, he then focused on the response of humans exposed to ionizing radiation for several decades, having accumulated much dose and genetic damage, making a sizeable background, and therefore a sizeable proportional risk, that presumably might be detectable in an epidemiological sense. The new dose is then added to the accumulated background damage in a manner that is additive to background. This is then represented as such in the Beninson figure (see Beninson, 1988- Fig. 6). This argument is of course, framed, as he noted, to be applied to the practical realm of devising a worker protection plan.

The arguments of Beninson fail to address important empirical genetic damage findings that challenge and possibly contradict his premises. For example, the failure to detect ionizing radiation induced genetic damage in multiple sensitive experimental systems even at doses from several hundred to many thousands fold greater than background is highly problematic (Russell, 1969). Epidemiological evidence also does not support Beninson's application of the additive to background as seen with the series of essentially flat excess risk

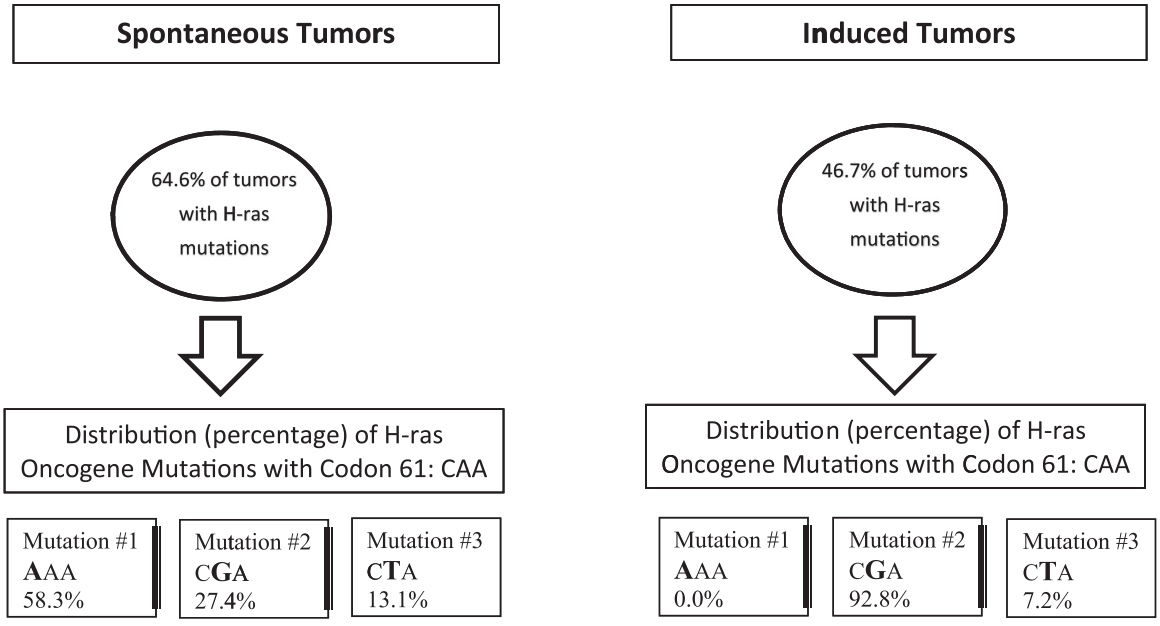

Fig. 3. Spontaneous vs NDEA induced liver tumors with H-ras oncogene mutations* in codon 61 of the B6C3F1 mouse (Source: Chen et al., 1993); marked boxes indicate oncogene mutations that differ between spontaneous and induced tumors. **Conclusion**: Induced tumors showed a significantly different mutation spectra as highlighted for mutations #1 and #2 than the spontaneous tumors. *Mutation percentages were summed across the three types of mutations in codon 61 for both spontaneous and induced tumors. Bolded, enlarged letters represent the location of mutation in codon in Figures 3–10.

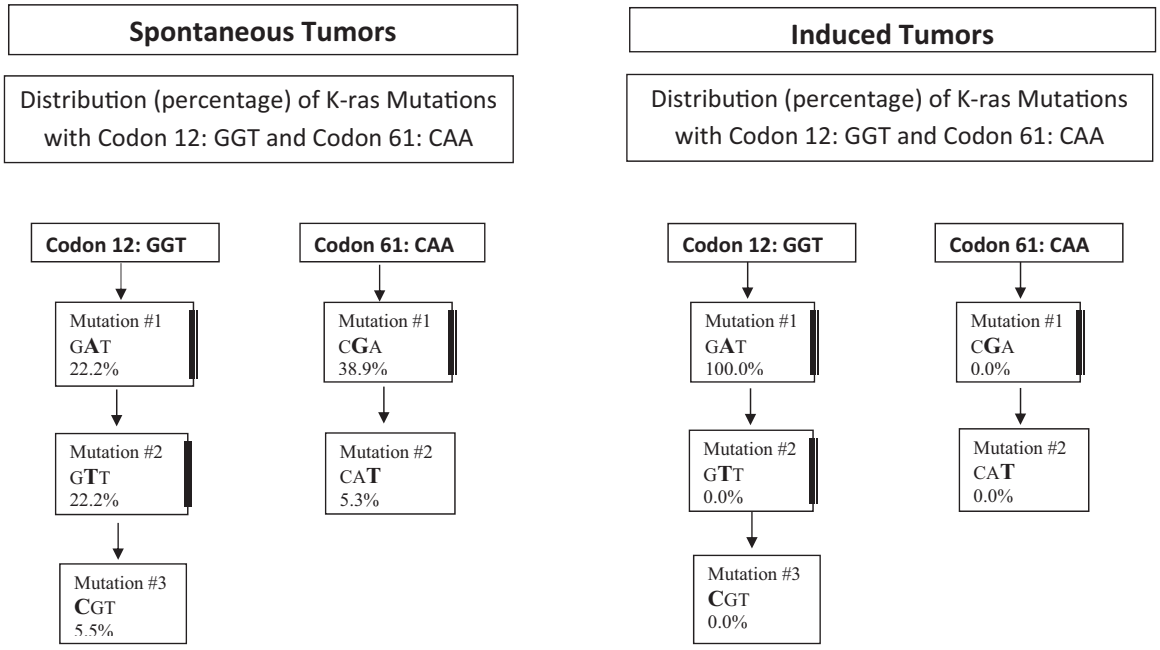

Fig. 4. Spontaneous vs NNK induced lung tumors with K-ras oncogene mutations* with codons 12 and 61 of the B6C3F1 mouse (Source: Chen et al., 1993);); marked boxes indicate oncogene mutations that differ between spontaneous and induced tumors. **Conclusion**: Induced tumors showed a significantly different mutation spectra as highlighted in codon 12 (i.e., mutations #1 and 2) and codon 61 (i.e., mutation #1) than the spontaneous tumors. *Mutation percentages were summed across the codons 12 and 61 for both spontaneous and induced tumors.

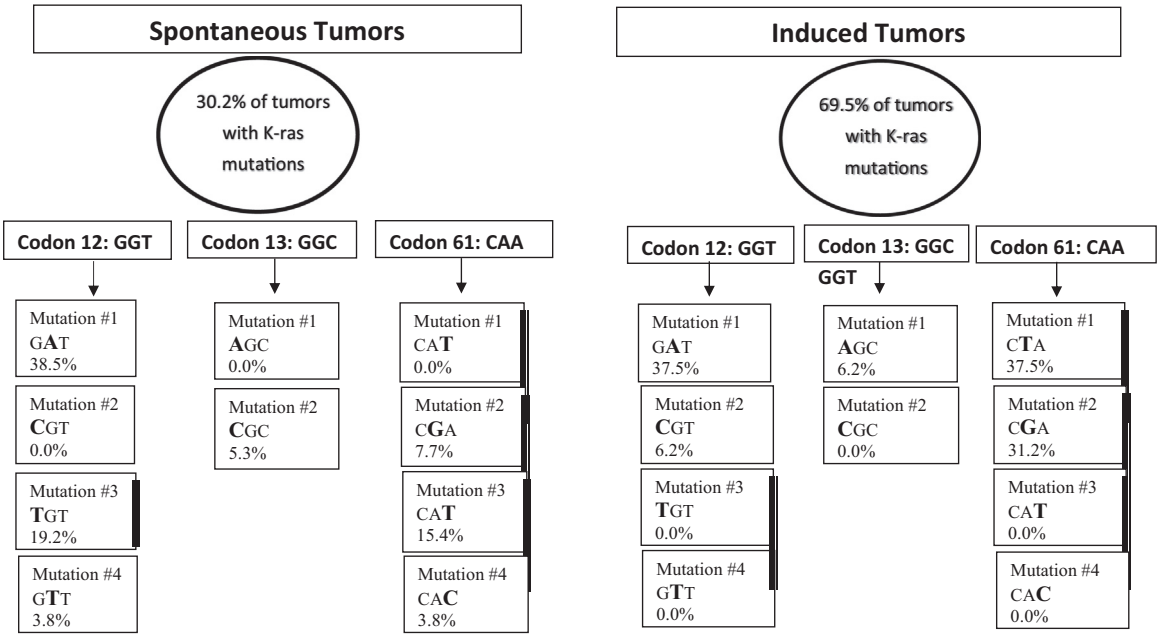

Fig. 5. Frequency/spectra of K-ras mutations* in lung tumors of B6C3F1 mouse study of ADBAQ (Source: Hayashi et al., 2001, Table 4, Page 426)[i] marked boxes indicate oncogene mutations that differ between spontaneous and induced tumors. **Conclusion**: Induced tumors showed a significantly different mutation spectra as highlighted in codon 12 (i.e., mutation #3) and codon 61 (i.e., mutations #1, #2, and #3) than the spontaneous tumors. *Mutation percentages were summed across the codons 12, 13, and 61 for both spontaneous and induced tumors.

responses across a broad range of radiation doses prior to reaching and exceeding a threshold (Ricci and Tharmalingam, 2018).

4.5. Tumor mutation complexity, hyperindividualistic tumor progression and challenges to additive to background

Of considerable relevance to the issue of additivity to background were a series of findings from large-scale efforts to systematically screen individual tumors for somatic mutation using human breast, colon, pancreas, and brain (i.e., glioblastoma) tumor samples (Sjoblom et al., 2006; Wood et al., 2007; Vogelstein et al., 2013). These tumor types were selected since they had a graded progression prior to reaching advanced, invasive stages. The guiding hypothesis was that there was a progressive mutational sequence that should be clinically related, reflecting an orderly, sequential pattern. The concept of cancer as a mutation-driven orderly disease as framed by Vogelstein et al. (2013) was born in the earlier multi-stage models of Armitage and Doll (1957) and Crump et al. (1976). The Vogelstein et al. studies are striking,

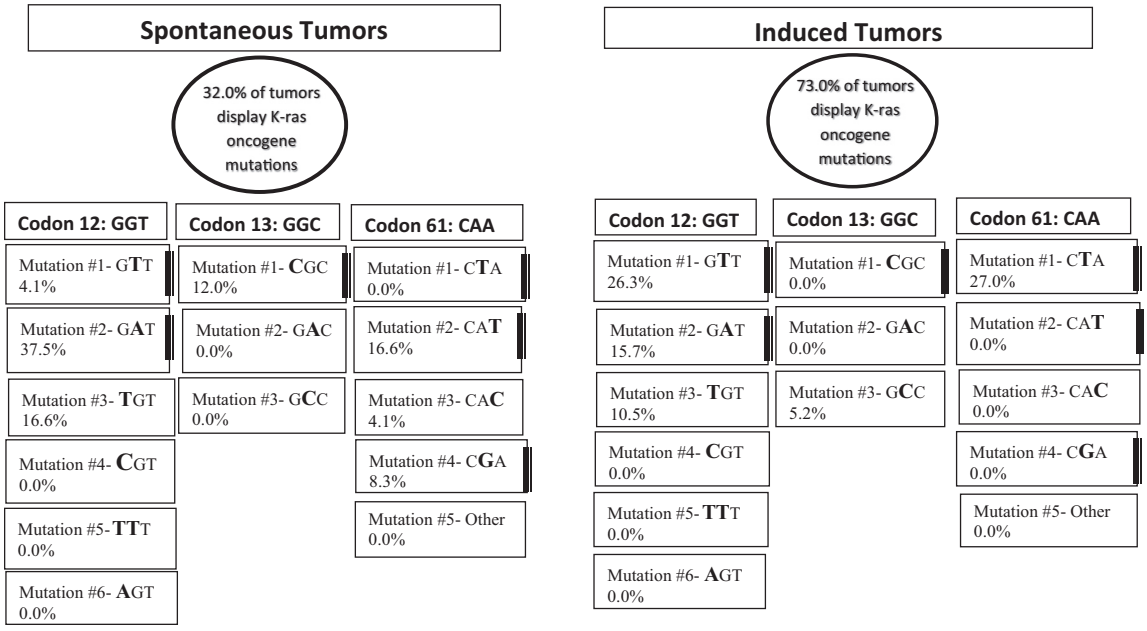

Fig. 6. Frequency and spectra of K-ras mutations* in controls and ozone exposed B6C3F1 mice (Source: Sills et al., 1995); marked boxes indicate oncogene mutations that differ between spontaneous and induced lung tumors. **Conclusion**: Induced tumors showed a significantly different mutation spectra as highlighted for Codon 12 (i.e., mutations #1 and #2), codon 13 (i.e., mutation #1) and codon 61 (mutation #1, #2, and #4) than the spontaneous tumors. *Mutation percentages were summed across the codons 12, 13, and 61 for both spontaneous and induced tumors.

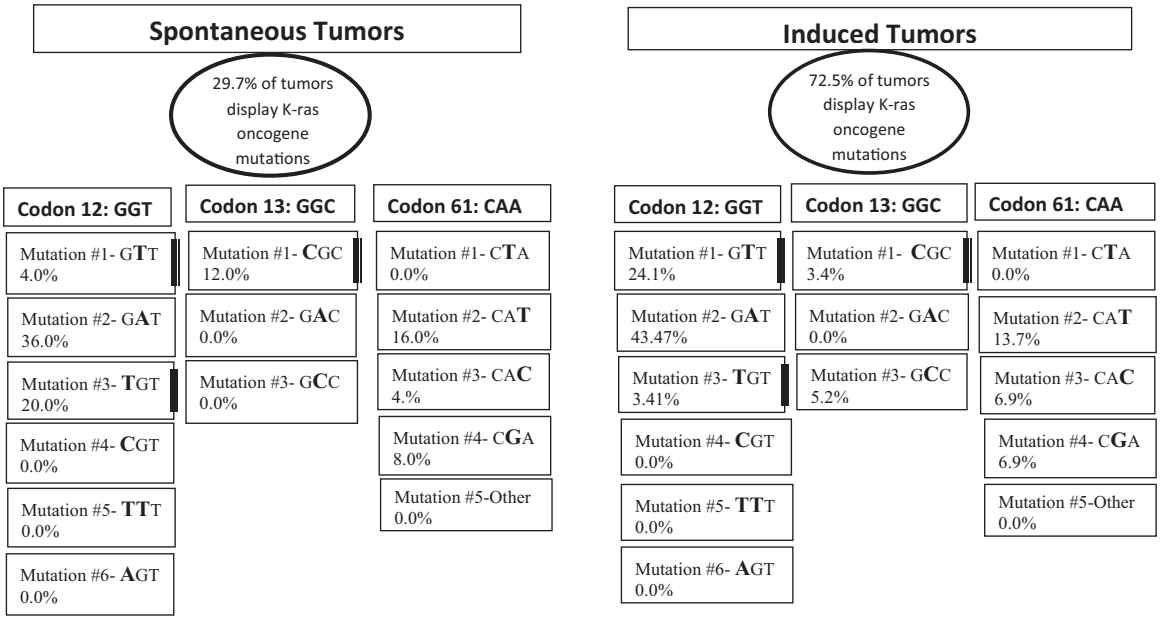

Fig. 7. Frequency and spectra of K-ras mutations in controls and vanadium pentoxide exposed B6C3F1 mice (Source: NTP Tech Rep 507, 2002; Ton et al., 2004, Table 2); marked boxes indicate oncogene mutations that differ between spontaneous and induced lung tumors. **Conclusion**: Induced tumors showed a significantly different mutation spectra as highlighted for Codon 12 (i.e., mutations #1, and #3), and codon 13 (i.e., mutation #1) than the spontaneous tumors. *Mutation percentages were summed across the codons 12, 13, and 61 for both spontaneous and induced tumors.

challenging this orderly progressive assumption. This perspective emerged from the substantial degree of complex intertumoral heterogeneity, and its progressively highly individualistic nature.

Driver mutations (i.e., genes that confer a selective growth advantage) appeared present for many cancer genes within the studied tumors. However, many infrequently altered non-driver passenger mutations (i.e., mutations with no direct or indirect effects of the selective growth advantage of the cell in which it occurred) when collectively combined make substantial contributions to tumor occurrence. In the case of breast cancer, the maximum number of mutated cancer genes (i.e., driver-like mutations) were six, along with about 50 possible passenger mutations. Nearly 30% of these tumors had a single driver mutation along with several passenger mutations, while about 5% had no identifiable oncogene plus tumor suppression gene mutations (Vogelstein et al., 2013).

While it is has long been established that cigarette smoking is a major risk of lung cancer, non-smokers still comprise about 10–15% of those displaying this disease (McCarthy et al., 2012). This relatively high occurrence of lung cancer in non-smokers offers the potential to evaluate the additive to background assumption within a population of

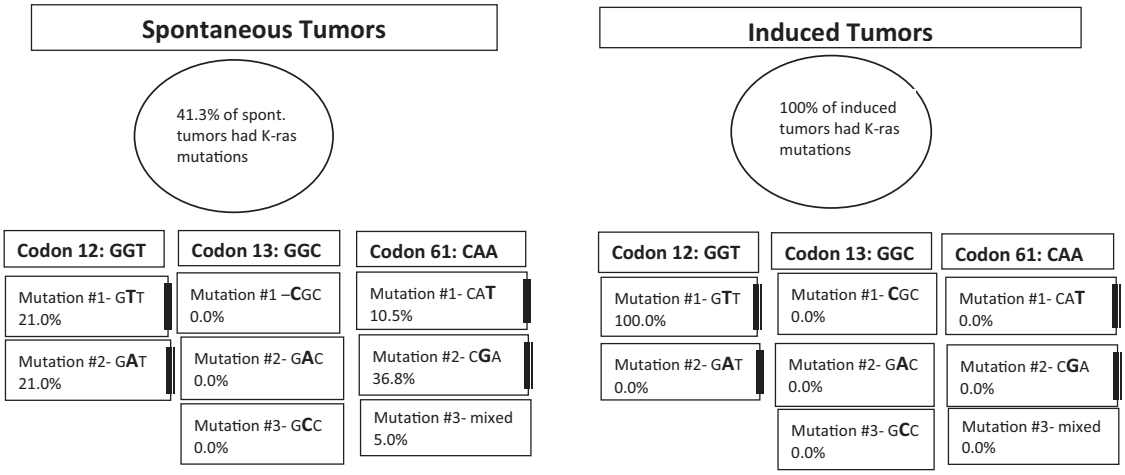

Fig. 8. Comparison of K-ras mutation spectra for spontaneous tumor and NNK-induced tumors in A/J lung tumors (Source: Matzinger et al., 1994; Ronai et al., 1993;Sills et al., 1999b); marked boxes indicate oncogene mutations that differ between spontaneous and induced tumors. **Conclusion**: Induced lung tumors showed a significantly different mutation spectra as highlighted for Codon 12 (i.e., mutations #1 and #2), and codon 61 (mutations #1 and #2) than the spontaneous tumors. *Mutation percentages were summed across the codons 12, 13, and 61 for both spontaneous and induced tumors.

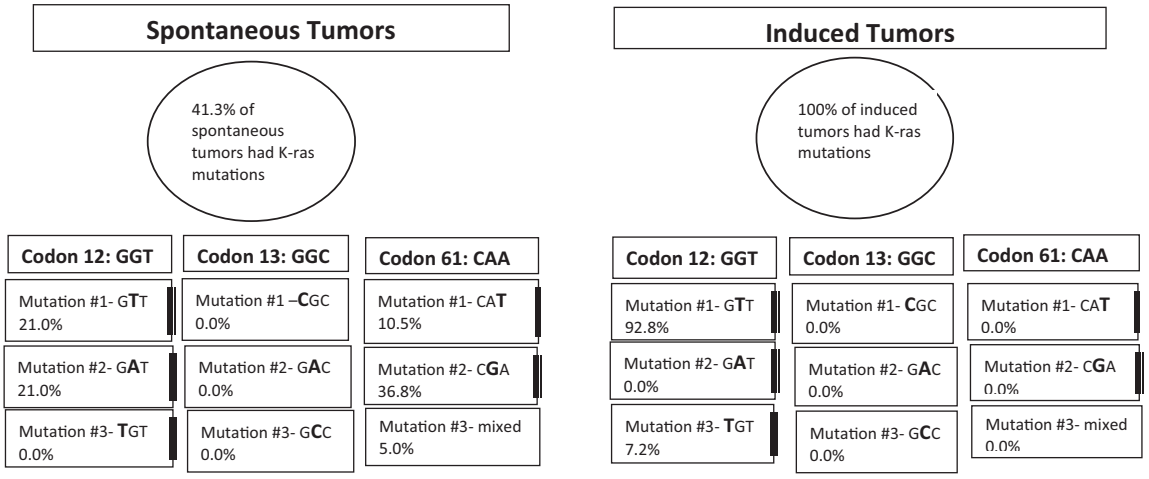

Fig. 9. Comparison of K-ras mutation spectra for spontaneous tumor and NNK-induced tumors in A/J lung tumors (Source: Matzinger et al., 1994; Ronai et al., 1993); marked boxes indicate oncogene mutations that differ between spontaneous and induced tumors. **Conclusion**: Induced lung tumors showed a significantly different mutation spectra as highlighted for Codon 12 (i.e., mutations #1 - #3), and codon 61 (mutations #1 and #2) than the spontaneous tumors. *Mutation percentages were summed across the codons 12, 13, and 61 for both spontaneous and induced tumors.

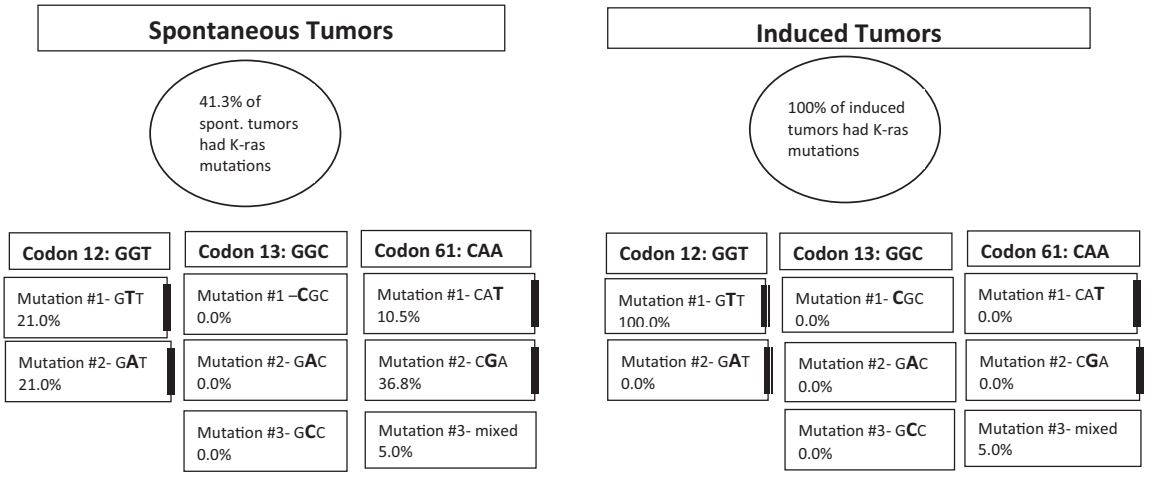

Fig. 10. Comparison of K-ras mutation spectra for spontaneous tumor and AMMN-induced tumors in A/J lung tumors (Source: Matzinger et al., 1994; Ronai et al., 1993; Sill et al., 1999b); marked boxes indicate oncogene mutations that differ between spontaneous and induced tumors. **Conclusion**: Induced lung tumors showed a significantly different mutation spectra as highlighted for Codon 12 (i.e., mutations #1 and #2), and codon 61 (mutations #1 and #2) than the spontaneous tumors. *Mutation percentages were summed across codons 12, 13, and 61 for both spontaneous and induced tumors.

Table 19
Differentiating spontaneous vs induced mutations: Perspectives offered by leading researchers.

References	Quotes
Antal et al. (2002)	"In lung tumors…the frequencies of many alterations were different in radiation-induced and spontaneous tumors, suggesting that different oncogenic pathways were activated during spontaneous and radiation-induced lung carcinogenesis of mice." Page 122
Hayashi et al. (2001)	"Evaluation of ras mutations in ADBAQ-induced forestomach tumors showed a high frequency of chemical-specific point mutations. Predominant H-ras codon 61 CAA to CTA transversions were detected in ADBAQ-induced but not spontaneous tumors. The A- > T transversions were not considered to be spontaneous because such mutations have not been detected in spontaneous forestomach tumors examined to date from $B6C3F_1$ mice." Page 427, left column
Hoenerhoff et al. (2013)	"While spontaneous and GBE-treated HCC in this study were morphologically very similar, in terms of their gene expression and mutation spectra, these tumors are actually quite different. HCCs in mice exposed to GBE were characterized by dose-dependent *Ctnnb1* mutations, with an increased incidence of deletions and multiple mutations per tumor in some high-dose animals. These features are markedly different from spontaneous HCC in this strain, which does not typically harbor deletion mutations or multiple mutations, and has a relatively low concurrent (0%) and historical control (2%) incidence rate of *Ctnnb1* mutations (Hayashi et al., 2003)." Page 12, Discussion section "…while spontaneous and GBE-treated HCC in B6C3F1 mice are very similar at the morphologic level, we have shown that the molecular alterations in GBE-treated tumors are very different from those seen in spontaneous tumors. These include unique alterations in *Ctnnb1* gene and protein expression, structure, and function;…" Page 14
Hoenerhoff et al. (2013) (Continued)	"Additionally, marked differences in global gene expression profiling, including overrepresentation of cancer signaling pathways, xenobiotic metabolism, and oxidative stress, shows that while spontaneous and GBE-treated HCC are morphologically indistinguishable, they may be distinguished based upon their transcriptomic profiles. This is of considerable importance when distinguishing between a background tumor incidence and chemically induced neoplasms, particularly in strains with moderate background tumor rates." Page 14
Hong et al. (2003)	"Spontaneous hemangiosarcomas from control mice lacked both p53 and β-catenin protein expression and ras mutations. Our data indicated that p53 and β-catenin mutations in the *o*-nitrotoluene-induced hemangiosarcomas and K-ras mutations and p53 protein expression in riddelliine-induced hemangiosarcomas most likely occurred as a result of the genotoxic effects of these chemicals." Page 227, Abstract
Hong et al. (2003)	"…in some neoplasms the profiles of activation mutations in ras genes or inactivating mutations in the p53 gene are specific for particular chemicals and differ from those detected in spontaneous neoplasms." Page 228, left column
Hong et al. (2015)	"In summary, there was a significantly elevated incidence of Kras mutations, accompanied by a lower incidence of Egfr and Tp53 mutation in lung tumors from mice and rats chronically exposed to CMD compared to SL tumors. The mutations detected in the Kras, Egfr, and Tp53 genes in CMD-exposed mice and rats clearly imply those genetic events are related to chemical exposure, since those mutations were not detected in the concurrent spontaneous tumors in mice and SL tumors from previous NTP studies in rats." Page 880, left column
Houle et al. (2006)	"…the chemically induced tumors exhibited a distinct shift in the P53 and H-ras mutational spectra compared to spontaneous tumors suggesting that benzene and EtO exposure induced mammary specific alterations predisposing female mice to mammary tumor development. The difference in mutation profiles between spontaneous and chemically induced neoplasms suggest that different mechanisms are likely involved." Page 756, Discussion, right column
Houle et al. (2006)	"A comparison…the cumene-induced tumors displayed specific p53 mutations. P53 mutations were observed only in exon 5 (24/27, 89%) and exon 7 (3/27, 11%). The mutations observed in the p53 gene in cumene-exposed mice clearly imply this genetic event is related to chemical exposure, since these mutations were not detected in spontaneous tumors." Page 725, left column
Hong et al. (2007)	"The predominant [K-ras] mutations [in B6C3F1 mice] in EO-induced Harderian Gland (HG) neoplasms consisted of GGC to CGC transversions at codon 13 (15/18, 83%) and GGT to TGT transversions at codon 12 (8/18, 44%). Neither of these mutations was found in spontaneous HG neoplasms (0/27)." Page 82, right column
Iizuka et al. (2010)	"To clarify how ionizing radiation induces mammary carcinogenesis, we characterized genomic copy number aberrations for γ-ray-induced rat mammary carcinomas using micro-array-based comparative genomic hybridization. We examined 14 carcinomas induced by γ-radiation (2 Gy) and found 26 aberrations, including trisomies of chromosomes 4 and 10 for three and one carcinomas, respectively, an amplification of the chromosomal regions 3g35g36, Sg32 and 7911 in two, two and four carcinomas, respectively. These aberrations were not observed in seven spontaneous carcinomas". Page 206, abstract
Iizuka et al. (2010)	"…we found that the majority of radiation-induced carcinomas, but not spontaneous ones, had some form of DNA copy number aberration, illustrating the association between copy number aberrations and radiation-induced rat mammary carcinomas." Page 212, left column
Imaoka et al. (2008)	"Gene expression profiles of three spontaneous and four radiation-induced carcinomas, as well as those of normal mammary glands, were analyzed by microarrays…we identified 50 genes that had different expression levels between spontaneous and radiogenic carcinomas… Thus, gene expression analysis distinguished between spontaneous and radiogenic carcinomas suggesting possible difference in their carcinogenic mechanisms." Page 349, Abstract
Imaoka et al. (2008)	"We show for the first time that radiation-induced rat mammary cancer is distinguishable from spontaneous ones according to their gene expression." Page 357, left column
Imaoka et al. (2008)	"This data indicates that spontaneous and radiogenic mammary cancer development involves distinct molecular and cellular mechanisms." Page 359, left column
Kawano et al. (1996)	"In the present study, K-ras gene mutations were identified in 72.2% of all the NNK-induced lung lesions examined and the major mutation was G to A transition at the 2nd base of codon 12, which clearly differed from the mutational pattern in untreated control mice [tumors]." Page 48, Right column.

(*continued on next page*)

Table 19 (*continued*)

References	Quotes
Liu et al. (2003)	"Total exon deletion did not exist in any spontaneous mutants but in γ-rays- and ENU-induced mutants. The proportions of deletion mutations were quite different between spontaneous mutants and induced mutants, and similar change of γ-rays- and ENU-induced mutants occurred at the hprt locus." Page 580, right column
Loktionov (1991)	"…A- > T transversions in the second position of codon 61 of Ha-ras were present only in liver tumors [CD− 1 mice] that developed in mice treated with DMBA…" Page 1189, Abstract
Lutz et al. (2002)	"The "additivity to background" concept which suggests low-dose linearity may also be questioned. The incremental DNA damage exerted by a genotoxic carcinogen may not always increase the probability of mutation. For instance, if exposure to a genotoxic agent induces DNA repair, and if the induced repair capacity also repairs background DNA damage, a non-monotonic (U-shaped) dose response might result. This type of dose response can no longer be dismissed (Calabrese and Baldwin, 2001a,b). It could also mean that a threshold-like dose response may in fact be U-shaped, and that the process of spontaneous carcinogenesis could even be slowed down at low dose of a "carcinogen"." Page 336, left column
Marxfeld et al. (2006)	"In this study, the histologically similar spontaneous and DMBA-induced adenocarcinomas (Figs. 1 and 2) could be separated by gene expression profiling." Page 159, right column
Mass et al. (1996)	"The distribution of these mutations is different from those in untreated animals from two previous studies." Page 1703 Note: These mutations are in lung tumors of A/J/ mice treated with benzo(b)fluoranthene
Nishimura et al., 1999	"To gain information on the possible role of Ras activation in development of thymic lymphomas in scid mice, we have examined both the frequency and the spectrum of Kras and Nras mutations in spontaneous and radiation-induced lymphomas. Neither activated Kras nor Nras genes were detected in spontaneous lymphomas, while Kras mutations increased in a dose-dependent manner in radiation-induced lymphomas." Page 142, Abstract
Schwartz et al. (1994)	"In the spontaneous mutants, most of the partial gene deletion mutations involved exons 4–6. In contrast, only exon 1 was deleted in the γ-induced partial deletion mutants so far, and in the α-induced spectra, deletion of exons 1–3 and 6–9 were found; no deletions of exons 4 or 5 were seen in either radiation-induced spectrum." Page 539, right column
Sills et al. (1995)	"…almost a third of the neoplasms induced by ozone exposure had an A- > T transversion in the second base of codon 61, a mutation not detected previously in 66 spontaneously arising lung neoplasms of B6C3F1 mice (19, 28) or in lung neoplasms from concurrent air controls." Page 1624, right column
Sills et al. (2001)	"…a high frequency of H-ras codon 61 CAA- > CTA transversion (10/41; 24%) was detected in chemically induced forestomach neoplasms, but none were present in the spontaneous forestomach neoplasms examined." Page 373, Abstract
Sills et al. (2001) (Continued)	"This is the first report in which the ras mutation frequency and spectra had been determined for spontaneous forestomach neoplasms of the B6C3F1 mouse… The exposure-related changes in ras mutations observed in this study were qualitative and quantitative, presenting both a different mutation spectrum from that of spontaneous occurring neoplasms of the forestomach, and an increase in the fraction of tumors containing ras mutations." Page 381 "The lack of H-ras codon 61 CTA mutations and the rare detection of K-ras codon 13 CGC mutations in the spontaneous forestomach neoplasms strengthens the hypothesis for a causal link between chemical exposure, ras mutations, and forestomach neoplasia following exposure to 1,3-butadiene and the structurally related analogs." Page 383
Ton et al. (2004)	"BMP-Induced Lung Tumors" "The predominant pattern of K-ras mutation detected in BMP-induced lung neoplasms consisted of G- > A transition (GGT- > GAT) at codon 12 (20/29)…it was significantly different from that observed in historical controls…" Page 17, right column
Ton et al. (2004)	"TNM-Induced Lung Tumors" "The predominant K-ras mutation detected in TNM-induced lung neoplasms consisted of G- > A transitions (GGT- > GAT) codon 12 (13/15) compared to none in spontaneous lung neoplasms from the inhalation control mice…" Page 18, left column

affected individuals that have been the object of substantial focused investigation with respect to this genomic landscape. Such investigations have revealed marked mutational differences in the tumors of smokers and non-smokers lungs with non-small cell lung cancer (Govindan et al., 2012). These include a different mutation spectrum, with C:G- > A:T predominating in smokers while in non-smokers C:G- > T:A predominated. There are also specific groups of mutations found in never smokers (i.e., EGFR mutations and ROS1 and ALK fusions) with KRAS, TP53, BRAF, JAK2, and JAK3 and mismatched repair gene mutations in smokers. These differences between smoker and non-smoker lung cancer subjects are therefore substantial and not linked to a few genes (Subramanian and Govindan, 2013). The findings indicate that induced and spontaneous lung cancers in humans are fundamentally different with respect to driver mutations.

These developments challenge both the biological foundation between spontaneous and induced tumors for driver mutations and the accompanying uncertainty of the additivity to background assumption in multiple ways, including the complexity of the process of carcinogenesis, its progressively hyperindividualistic tumor development, the presence of differing driver mutations between spontaneous and induced tumors, and the difference in the number and sequence of contributing mutations.

Given this complexity and the growing recognition of the different mutational signatures, there is no convincing biological justification for the use of the additive to background assumption as a default feature for cancer risk assessments. It is possible to speculate that a certain proportion of tumors may have some unknown mechanistic overlap, but how these occur and what an overlap means biologically, and how it may affect tumor progression for spontaneous and induced tumors is unknown. The closest linkage of these recent tumor complexity developments (Lin et al., 2007; Jones et al., 2008; Killela et al., 2013; Vogelstein et al., 2013) to the present additive to background

assessment is that spontaneous and induced tumors have significant mutational differences which are likely to contribute to the individual tumor progression and hyperindividualisticity making the comparison between spontaneous and induced tumors highly tenuous and excessively speculative.

4.6. Additive to background for cancer vs non-cancer endpoints

The issue of additive to background was formulated to address low dose cancer risk assessment concerns. The validity of the additive to background assumption in this cancer risk assessment context is the focus of the present paper. However, there has been a debate over whether the additive to background concept would also apply to non-cancer endpoints. While this debate does not seem unlike the cancer risk assessment issue with discussion focusing on the occurrence of ongoing disease processes and heterogenicity within the human population, Rhomberg et al. (2011) argued that cancer risk from genotoxic agents is qualitatively different with the discrete and stochastic features of induced mutations, which is assumed to originate in a single cell, with subsequent monoclonal expansion. The cancer disease process is profoundly different from how most non-cancer effects seem to occur. However, even with such markedly different aspects to the disease processes for cancer and non-cancer effects, the additive to background assumption still would require that non-cancer endpoints occur via the same mechanisms for the inducing agents and the background disease processes. As seen in the present paper, advances in molecular biology have revealed that different mechanisms seem to be the rule rather than the exception. If this is the case for non-carcinogen endpoints, then current theoretical arguments made on behalf of this hypothesis (White et al., 2009) (see Crump, 2017 and Bogen, 2017 debate) would be challenged to provide the specific mechanistic data.

5. Conclusions

The question proposed in this paper is that posed by Hoel (1997), that is:

> "Whether or not the original simple idea of background additivity is consistent with today's biology and whether the concept, if true, has any value for quantitative risk estimation."

1. Multiple, complementary and converging lines of evidence indicate that the "original simple idea" of additive to background for the induction of tumors that became incorporated into regulatory agency (i.e., EPA) cancer risk assessment is **not** consistent with modern molecular biology and toxicology.
2. Studies from 45 carcinogens, over a dozen animal models and a broad range of organ- specific tumors contradict the additive to background assumption that induced and spontaneous tumors act via identical mechanisms.
3. Detailed genetic landscape studies of multiple human tumors, based on detailed assessments of driver and passenger mutations, support these animal bioassay studies by their illustration of the progressive hyperindividualistic tumor development.
4. Carcinogen treatment in the chronic bioassay commonly reprograms biological processes markedly altering oncogene activities, mutation frequency and the mutation spectra of normally developing spontaneous tumor development processes. These changes can profoundly alter spontaneous tumor incidence and phenotype and the control group reference for carcinogen treatment evaluation. These findings indicate that the traditional control group may not be a useful standard for comparison, since the biological/genetic reprogramming, due to carcinogen treatment, can significantly change the "background" tumor expectation observed in the treatment group. These findings challenge the validity and utility of the additive to background and the independent of background concepts.
5. While it is now known that the induced tumor mechanisms are very likely to be profoundly different than spontaneous tumor mechanisms, a principal risk assessment question is what proportion of the overall cancer mechanistic progress may overlap between these tumors and to what extent would any degree of overlapping affect the risk estimation. Since this area is extremely speculative and complex, it is not possible to provide specific qualitative and quantitative estimates. It would also not be possible to extrapolate information from one tumor type to another.
6. The present assessment reveals that the original additive to background assumption is not supported by modern molecular biology and toxicology. Numerous findings contradict the additive to background hypothesis of identical mechanisms for spontaneous/induced tumors. There is also a lack of information concerning whether and to what extent there may be some degree of mechanistic overlap between the spontaneous/ induced tumor processes or even if this is an appropriate question. It is not known if similar partial pathway(s) occurring at different time(s) affect the cancer process, time to tumor, how this may be affected by dose and any biological implications if mediated by driver or passenger mutations in unique combinations and/or sequences.

Trying to formulate a modified additive to background policy on a hypothetic basis of what proportion of assumed processes might be identical for spontaneous/induced tumors adds an even more uncertain perspective which should caution against governmental risk assessment policy/procedures on such matters. Furthermore, observations that carcinogen treatment can reprogram spontaneous tumor endpoints may have significant risk assessment implications, contradicting the additive to background and independent of background assumptions. Neither the continued use of the additive to background assumption/procedure or a switch to an independent of background assessment process appears justified. In their absence this would return the evaluative process to a direct experimental comparison without underlying hypothetic assumptions.

Declaration of Interest

None.

Funding Sources

This work was supported by the US Air Force Office of Scientific Research (AFOSR FA9550-13-1-0047) and ExxonMobil Foundation (S18200000000256). The U.S. Government is authorized to reproduce and distribute for governmental purposes notwithstanding any copyright notation thereon. The views and conclusions contained herein are those of the author and should not be interpreted as necessarily representing policies or endorsement, either expressed or implied. Sponsors had no involvement in study design, collection, analysis, interpretation, writing and decision to and where to submit for publication consideration.

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