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The impact of dose rate on the linear no threshold hypothesis

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ABSTRACT

The goal of this manuscript is to define the role of dose rate and dose protraction on the induction of biological changes at all levels of biological organization. Both total dose and the time frame over which it is delivered are important as the body has great capacity to repair all types of biological damage. The importance of dose rate has been recognized almost from the time that radiation was discovered and has been included in radiation standards as a Dose, Dose Rate, Effectiveness Factor (DDREF) and a Dose Rate Effectiveness Factor (DREF). This manuscript will evaluate the role of dose rate at the molecular, cellular, tissue, experimental animals and humans to demonstrate that dose rate is an important variable in estimating radiation cancer risk and other biological effects. The impact of low-dose rates on the Linear-No-Threshold Hypothesis (LNTH) will be reviewed since if the LNTH is not valid it is not possible to calculate a single value for a DDREF or DREF. Finally, extensive human experience is briefly reviewed to show that the radiation risks are not underestimated and that radiation at environmental levels has limited impact on total human cancer risk.

1. Introduction

Radiation standards are set primarily based on human epidemiology studies with a focus on the A-bomb survivors. These data are evaluated using the Linear-No-Threshold Hypothesis (LNTH) to derive risk factors. This event exposed a large human population to graded radiation doses delivered in a very short time. Serious efforts made it possible to estimate individual doses and to relate the cause of death and the frequency of disease, especially cancer to the dose of that individual. The exposed population was compared to a carefully matched control group not exposed to the bomb. Such exposures as well as studies on radiation therapy patients have been shown to increase cancer frequency [1,2,3,6]. These studies also suggest an increase in several non-cancer endpoints such as cardiovascular disease [4] cataracts [5] and stroke [6,7]. It is important to note that the two populations compared, those exposed to the bomb and those not exposed have very different life experiences. In addition to the radiation from the bomb, the exposed population was exposed to trauma, blast, burns and stress, all of which may contribute to the excess cancer observed. Most of the excess cancers were in the highest dose groups with little significant difference seen in those with lower doses.

To evaluate the scientific validity of the Liner-No-Threshold Hypothesis (LNTH) for radiation risk assessment, it is critical to understand and account for, the substantial influence of both dose and dose rate with respect to potential adverse effects on biological systems. Since the first demonstration of the impact of radiation on biological organisms it was recognized that when the same dose of radiation was delivered over a short period of time it was more effective in producing biological changes than when it was given over a longer time. Consideration of both have been involved in regulation of radiation exposure to protect workers and the public from harm. The use of the LNTH in standard setting has included a Dose-Dose Rate Effectiveness Factor (DDREF) that recognizes the responses to low doses and low-dose rates are less effective in increasing risk than single acute exposures (National Council for Radiation Protection and Measurement [8,9] United Nations Scientific committee on the Effects of Atomic Radiation [10,11] and National Research Council/National Academy of Sciences [12]. The recognition of the influence of dose and dose-rate in the low dose range has resulted in a range of values for the DDREF, for example 1.5 [12], 2.0 for the ICRP 2007, and the French Academy suggested that at low doses and dose rates the DDREF may be very high [13]. Recently the German Commission on Radiological Protection (Strahlenschutzkommission [SSK] suggested that the DDREF be abolished, that is that it be set at 1.0 [129]. If, as suggested by the German group, the DDREF is 1.0, the LNTH is applicable in all situations. In addition, it would be accurate to use collective dose to estimate risk regardless of dose rate and there needs to be no consideration of the role of dose rate on risk. The Health Physics Society strongly opposes this practice and suggests that collective dose should play little role in risk assessment [14]. Using collective dose, it is possible to sum many small doses or doses delivered at a low-dose rate to a large population and derive a large total collective dose. This collective dose combined

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with a risk factor derived from a single acute exposure, has been used to calculate a predicted number of excess cancers from such treatments as CT scans [15].

To better understand risk associated with low-dose rate exposure it is important to define the terms used. The use of DDREF has always been considered necessary for converting cancer risks derived at relatively high and acute doses, primarily from epidemiological studies of the A-bomb survivors [1,2,3,6] to calculate risks in the low dose (< 100 mGy) and dose-rate (< 5 mGy/h) range. However, it has been proposed that it is more appropriate to consider both a low dose effectiveness factor (LDEF) and a dose-rate effectiveness factor (DREF) for risk estimate calculations [16,17].

The LDEF is calculated as the ratio of the slope of the linear extrapolation from a point on the linear quadratic (LQ) curve and the slope of the linear component of this LQ curve. Thus, for acceptance of this approach, it is essential to establish the dose-response relationship for the induction of cancer and show that it fits a LQ function. For leukemia in the A-bomb data this seems to be the case while there are still uncertainties associated with the effects of low doses for solid cancers which have been postulated to be linear [3]. Recent studies suggest that using the shape of the dose response curve to estimate a dose rate effectiveness factor does not fit the data [18]. Comparing slopes of dose-response relationships derived for high and low-dose rate exposures provides more accurate assessment of the DDREF. This has been demonstrated for both human data [18] and for large mouse studies [19]. These studies all demonstrated DDREF values that were greater than 2.0 and suggested the need to reevaluate the current values used for standards. Calculation of a DDREF assumes that the dose-response relationship in the low dose region is linear. If it is not linear then it is not possible to calculate a single value for a DDREF.

The more acceptable way to calculate a DREF is by comparing the ratio of the slope of the dose response for acute doses to that for the same doses delivered at a low-dose rate [16,19,20]. With this approach it is possible to evaluate the influence of high doses delivered at a low-dose rate such as deposition of internally deposited radioactive materials in Beagle dogs [21,22].

The development of modern molecular and cellular biology combined with new technology made it possible to measure biological responses in the low dose region that were not possible in the past. The application of these techniques to low doses and dose-rates by the Department of Energy Low Dose Radiation Research Program (http://lowdose.energy.gov). The program made it possible to measure radiation responses in the low dose and dose-rate region [23]. Similar approaches have been used in the European Union (MELODI, Epirad bio, Store and DoReMi) (http://www.doremi-noe.net) the Japanese research IES (http://www.ies.or.jp/index_e.html) and the Korean Society for Radiation Bioscience (http://www.ksrb.kr/english/into/intor_01.asp). This research demonstrated the need for major paradigm shifts in the field of radiation biology [24].

- Hit theory must be replaced by cell/cell communication and the role of the response of the whole organ not single cells as critical for in cancer induction. Many multiple level biological organization changes are required to induce cancer [25].
- The mutation theory of cancer and the role of mutations in the induction of cancer demonstrate that mutations play a role in cancer induction but alone may not be sufficient to produce this complex disease. The single mutation theory of cancer must be questioned.
- Extensive research demonstrated adaptive protection mechanisms at many levels of biological organization [26]. Marked differences in the cell and molecular responses observed in the low dose and dose-rate region compared to those seen in the high dose region demonstrated that the LNTH cannot be supported by new cell and molecular data [27].

This manuscript is organized to present data at all levels of biological organization. The data at the cell and molecular level are presented first to provide a mechanistic basis for the manuscript. Experimental animal data was required to link the mechanistic data to real cancer data with all defense systems in place. To provide a better basis for the cancer risks in humans experimental dog data is used. Finally, human data where large populations were exposed to low doses are briefly reviewed. This brief explanation helps the reader follow the flow of the manuscript.

Using modern data, the influence of dose-rate has been evaluated at the cell and molecular level on the key events in the critical pathways to the induction of cancer [20]. This approach is similar to how the Environmental Protection Agency (EPA) establishes regulatory limits for many chemicals [28–30]. This research resulted in a DREF of much greater than one demonstrated for many of these important changes in the progression of normal cells to become cancer [20].

Observations on the influence of dose rate in whole animal studies have been published for many years. Without exception the protraction of radiation dose results in less biological change than observed with a single acute exposure, regardless of the endpoint measured [21,22]. The majority of the data show large thresholds below which increased cancer frequency cannot be detected.

This experimental data is supported by low-dose rate exposure to human and taken as a whole supports a DDREF much greater than one and shows that collective dose is not a useful concept.

2. Results

2.1. Molecular, cellular and tissue data

2.1.1. Background information

Radiation standards have, for the most part, been established based on human epidemiology data using the LNTH extrapolation from the high dose data combined with a DDREF factor for the low dose and dose rate exposures. Data from molecular, cellular and tissues have been evaluated but had little impact on standards in the past. As the level of sophistication in these fields has developed the power to measure both adverse and beneficial biological changes in the low dose region has increased. It is now possible to measure the influence of both dose and dose-rate on the critical steps needed to change a normal cell into a cancer. These steps have been summarized, published and updated [25] and called the Hallmarks of Cancer (Fig. 1). These changes are observed in cancer and seem to be essential for the evasion of defenses, progression, development and metastasis in cancer production.

Using these Hallmarks as a guide, studies on the role of dose rate on molecular, cellular and tissue level changes in key events along the critical pathways needed for the development of cancer have been conducted and reviewed [20]. When comparing the responses of these sensitive molecular, cellular and tissue systems following exposure to high and low-dose rates of low linear energy transfer (LET) ionizing radiation three major categories of responses were observed and are discussed in the following sections.

- First, there are many publications where single or small numbers of doses were delivered at either a high or low-dose rate. In these studies, a marked response was observed following high dose rate with little or no response for the same endpoint exposed to the same dose but delivered at a low-dose rate. Since the response to the low-dose rate is zero or not detected it is not possible to directly derive a DREF. However, these studies suggest a very high DREF. To estimate DREF from any study one divides the response to the high dose rate by the response to the low-dose rate, in many of these studies, zero. Dividing any value by zero results in infinity, making it impossible to assign a numerical value.
- Second, studies were conducted where complete dose-response data were available following exposure to high and low-dose rates. For such studies the linear slopes of the dose-response relationships

were compared and the slope of the response following a high dose rate exposure was divided by the slope of the low-dose rate and a positive DREF factor derived. In most cases these studies supported a DREF value much greater than one with some with values as high as 30.

- Finally, there were several studies where the exposure to low dose or dose rate resulted in a decrease in the molecular, cellular or tissue responses below that observed for the controls. For such endpoints a negative or protective DREF value would be derived. This suggests a protective effect for low dose and dose-rate and such data would require the use of negative values in any model to describe risk [31,32].

Much of the early data on the biological responses induced by low doses of radiation were derived from the U.S. Department of Energy Low Dose Research Program and have been summarized in a book [27]. This book and other publications provides insight on the data at the molecular, cellular and tissue level [20,27]. A brief summary of the three types of studies described above is provided in the following sections.

2.2. Molecular and cellular changes

2.2.1. A single dose delivered at a high vs low-dose rate

Single or small numbers of different doses were delivered at a high or low-dose rate and cell and molecular measurements were made to evaluate the influence of dose rate on biological responses. These measurements were made at several different levels of biological organization. For many endpoints it was possible to measure a response following a low dose given at a high dose rate, but no response was detected when the same dose was delivered at a low-dose rate. If there is zero response following exposure to low-dose rates and the biological response following acute exposures is divided by zero or the response following low-dose rate exposure this results in infinity which has little meaning. Perhaps if the doses would have been higher for the chronic exposure a response could have been detected. This was seen for DNA damage where low doses given at a low-dose rate resulted in no detectable response while the same dose delivered as an acute exposure resulted in a readily measurable response [33]. This could be related to the non-linear formation of DNA repair foci where, per unit of dose, there were many more foci after low doses than were observed after higher doses [34]. However, these data are in direct conflict with data which demonstrated that at low doses, the dose required to trigger repair of DNA was not activated and no repair was detected [35,36]. More research is needed to resolve these differences in DNA repair in the low dose and dose-rate region.

Chernobyl created an interesting experimental setting. The dose from the accident in some locations was very high (greater than 1.0 Gy/year) but the dose was delivered at a low-dose rate. Attempts were made to measure mitochondrial DNA damage in bank voles exposed to this radiation environment and none was detected. However, if the same dose was delivered as an acute exposure marked damage was detected [37]. Studies were conducted to detect the induction of micronuclei in the bank voles and the same result found. No response to the low-dose rate exposure with a marked response following high dose rate [131]. Additional studies were conducted with C57B/6 and BALB/c mice to determine if this response was related to the evaluated animal species with the same result [38], which supported the earlier work on micronuclei [39]. These measurements suggested that the dose rate effectiveness factor is very large.

2.2.2. Complete dose response, high and low-dose rate (Response higher than controls)

The second type of studies reviewed [20] had data that had complete dose-response relationships with both high and low dose-rates both of which resulted in an increased level of cell and molecular change above that seen in the controls. For these data sets, it was possible to fit the data and compare the linear slopes of the dose-response relationships to derive a dose rate effectiveness factor (DREF). These data also represent a range of different levels of biological organization. At the DNA damage and repair level the frequency of γH2AX foci was measured as a function of dose rate over a range of doses up to 5.0 Gy. Following both high and low-dose rate exposure there was a linear increase in the frequency of γH2AX foci. When the slopes of the lines were compared it resulted in a very large DREF, about 30.0. Such data makes a strong case that the LNTH is not valid and that collective dose cannot be used when the doses are delivered at different dose rates.

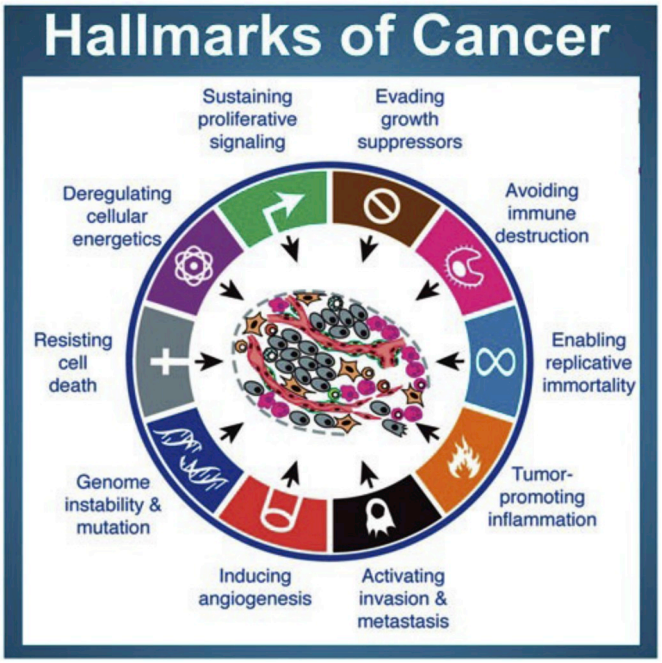

Fig. 1. The Hallmarks of Cancer demonstrates the changes that must take place for a cancer to be expressed. These changes range from the molecular to the whole tissue and illustrate that there are multiple changes needed to result in cancer. Most of these are not related to a simple mutation but involve tissue and whole animal responses [25].

Changes in gene expression and alterations in metabolic pathways have also been evaluated as a function of dose-rate. It was determined that the gene expression changes as a function of dose [40–42] and that the types of genes expressed at high doses are different from those produced following low-dose rates. Many of the genes activated at by low dose and low-dose rate exposures were involved in processes that seem to be protective while many of the genes activated after high doses are responses to damage. It was demonstrated that changes in oxidation/reduction pathways were modified as a function of dose and dose rate [44]. Changes in MnSOD and NF-kB were noted with low doses being suggestive of protective changes and high doses as damaging [45,46]; [123]). Many of these studies were summarized by Ref. [47]. It was also determined that different sets of genes were activated as a function of dose rate, time after exposure, and tissue types and that many of these genes were related to the induction of stress responses [48]. Extensive research has suggested that changes in gene expression can also be used as a biomarker of radiation dose for either high or low-dose rate exposure [49–51]. Many of these studies suggest that the LNTH is not valid with a DREF greater than one but none of them are useful in estimating a value for DREF.

Dose-response relationships have been measured for the induction of chromosome aberrations in the liver of Chinese hamsters after exposure to both acute and protracted whole-body exposure to ^{60}Co or

protracted exposure from internally deposited radioactive materials. Since the dose-response relationship was linear for the protracted exposure and non-linear for the acute exposure it was not possible to derive a single value for a DREF. If the response at a single dose, such as 1.0 Gy was used as the basis for the comparison, values of about 2.0 were derived with the values increasing as dose increased [52]. Similar values (1.8) were derived for chromosome aberrations in human blood lymphocytes given dose rates that varied from 400 to 1.9 rads/hour and comparing the responses again at a total dose of 1.0 Gy [53]. Using advanced chromosome painting techniques, it was possible to derive an alpha coefficient for the induction of chromosome translocations, the aberrations thought to be the most important in the induction of cancer [54]. This linear coefficient makes it possible to compare acute and chronic exposures. Using these advanced chromosome techniques [55] was possible to estimate DREFs which ranged from 2.0 to 3.0 depending on the dose used for the comparison.

Several studies focused on induction of chromosome aberrations in Chinese hamsters which were injected with ^{90}Sr-^{90}Y. In these studies, the aberration frequency increased as a function of dose rate [56,57]. It was postulated that the dose accumulated in each cell cycle was responsible for the damage observed at metaphase in these rapidly dividing cells [20].

Dose-response relationships were observed and measured as a function of both high and low-dose rate and the frequency of micronuclei in lung fibroblasts showed a linear dose-response relationships (Fig. 2). This makes it possible to divide the slopes of the lines and directly derive a DREF. When the acute exposure response was compared to that following a 4 h protraction of the same dose (dose rate 0.96, 1.95 and 2.9 Gy/hr) the DREF was 2.6, this value increased to 6.0 as the exposure time was increased to 67 h and the dose rate decreased (dose rate 0.059, 0.12, and 0.17 Gy/hr) [58]. Such data demonstrate that collective dose cannot be used and that dose rate is very important. These data do not provide scientific support for the LNTH without the use of a DREF.

For cell killing, measured as the ability to form colonies following exposure, the impact of dose rate was very dependent on the genetic background of the cells with a range from 1.0 to 10.0. With the same genetic background, it was determined that the DREF was greater than 10.0 as the dose rate continued to decrease [59]. Thus, cell killing shows a marked dose rate effect with repair in the low dose and dose rate range providing additional data that does not support the use of collective dose or the LNTH.

2.2.3. Complete dose response, high and low-dose rate (Response to low dose lower than controls)

The third type of response found as a function of low dose and dose rate exposures was when the radiation resulted in a decrease in the response below that observed in the controls.

Programmed cell death or apoptosis plays a critical role during fetal development as cells die during differentiation to produce organs. Recently it has been shown that apoptosis is also induced by exposure to ionizing radiation [60]. A critical observation about apoptosis is that it can be induced differentially in transformed cells resulting in a higher frequency of death. This differential cell killing results in a decreased risk following exposure to low doses of radiation with a decrease in the number of transformed cells. In the low dose and dose region of the dose-response relationship the frequency of transformed cells undergoing apoptosis was demonstrated to be higher than normal cells [61,132] . This selective apoptosis of transformed or damaged cells may result in a decrease in cancer risk and can be used to explain why low doses of radiation has been shown in some studies to reduce both cell transformation [62,63] and mutation frequency [64]. Such observations cannot be ignored and provide direct evidence that at low doses and dose rate the risk is either not measurable or may in fact be protective.

Very low doses delivered at a high dose rate have been shown to decrease the frequency of transformed cells to values below that observed in the control cells [62]. When the dose was delivered at a low-dose rate the frequency of transformed cells remained below the level observed in the controls for total doses as high as one Gy [65]. This is illustrated in (Fig. 3). It is important to note that each experiment on cell transformation must be related to its own control value since long term culture also increased the cell transformation frequency.

For transmitted mutations in mice it was determined early in the history of radiation biology that protracted exposures were less effective in producing mutations than single acute exposures to the same dose [66,67] with a DREF of 3.0 suggesting a non-linear dose response. Further research was conducted to determine the type of mutations that were produced by the radiation and it was determined that most of the dose-rate effect was seen for large deletions, such as those mostly produced by ionizing radiation, again with a DREF of about 3.0 [68]. When other types of DNA changes, which resulted in transmitted mutations were evaluated it was determined that the high and low-dose rate resulted in similar frequency of mutations suggesting that the DREF would be 1.0 for mutations that did not include large deletions and gross rearrangements [68].

2.3. Animal studies

2.3.1. Rodents

Moving from the molecular, cellular and tissue levels of biological organization it is critical to evaluate the whole animal responses to low-dose rate radiation exposures. Extensive research has been conducted using animals to demonstrate the influence of dose, dose rate and dose distribution on the induction of cancer. This manuscript starts by discussing rodent studies, which demonstrated that whole-body exposure to low-dose rate was less effective than high-dose rate in producing several different types of cancer [69]. These data, along with other information, were used by BEIR VII to estimate a DDREF of 1.5. There are several problems with rodent studies. First, many rodents die of specific diseases at early times so that the limited lifespan does not provide the needed latent period for the observation of radiation induced cancer. Second, the type of cancer produced by radiation is dependent on the rodent strain. It seems that each type of laboratory rodent produces a unique cancer type following radiation exposure so that they do not have the wide range of different cancers seen in humans. In addition, some rodents are very resistant to radiation while others are more sensitive. For example, rats develop a high frequency of lung cancer when exposed to radon while hamsters do not have a dose related increase in lung cancer. Some strains of mice are very resistant to radiation induced cancer, C57B/6 while other strains BALBc are more sensitive. These differences make extrapolation of cancer risk in rodents across species to humans almost impossible. Rodent studies conducted at the Argonne National Laboratory have been published and after careful reviews [19]. confirmed that the data on radiation induced life shortening could not be fit to a linear quadratic function used to evaluate the influence of dose rate in human studies (BEIR VII). To evaluate the influence of dose rate it is important to compare the slopes of dose-response relationships. The animal data all support the use of either a negative or high DREF suggested that dose rate has a marked impact on cancer frequency. These high values for DREF do not support the LNTH and make the use of a dose rate factor of one suggested (German Commission on radiation Protection 2016) non-supportable by basic science. Thus, there are dose rate effects at every level of biological organization from the molecular to experimental animals.

2.3.2. Dog experiments

Many years of research using the Beagle dog as the experimental animal, have been conducted and published on the health effects of internally deposited radioactive materials. The dog makes a good experimental animal. It has a long-life span and makes studies on latent period useful. The dog develops a spectrum of tumor types that are

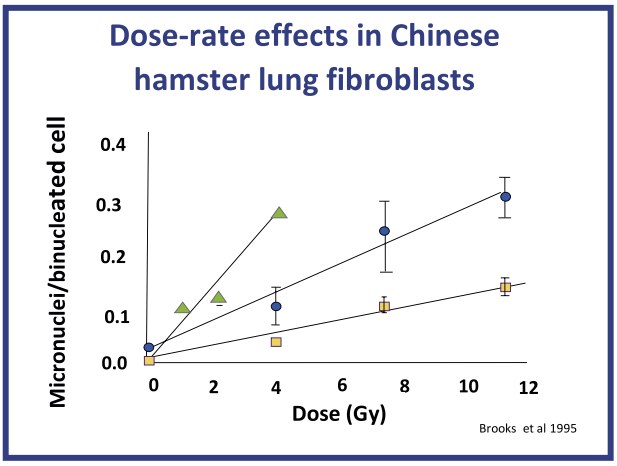

Fig. 2. This figure plots exposure in Gy against the frequency of micronuclei in lung fibroblasts. The figure demonstrates that low-dose rate exposures are less effective in producing chromosome damage than acute exposure [58].

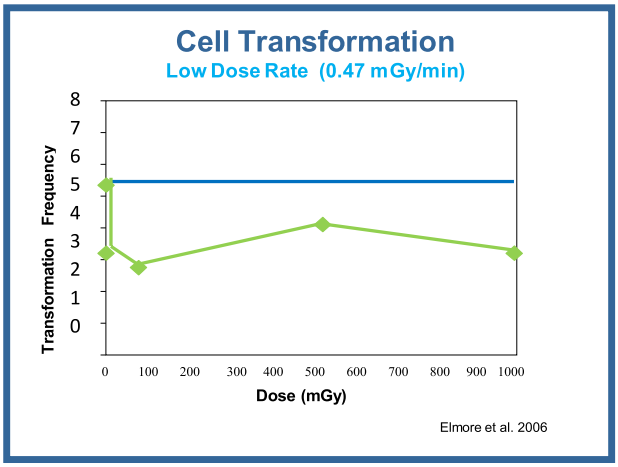

Fig. 3. Cell transformation frequency is plotted as a function of radiation dose. The dose was delivered at a low-dose rate 0.47 mGy/min with doses up to 1000 mGy (1.0 Gy). These low-dose rate exposures resulted in a depression of the cell transformation frequency below that observed in the controls [65].

similar to those seen in humans. Following single acute exposure to radiation the induced cancer frequencies are similar to that observed in humans [70]. The dog is large enough that each animal can be treated as a clinical subject so that important biological changes like pulmonary function [133], blood counts [71], blood chemistry [72] and histopathology and tumor type [73] can be measured as a function of time after the exposure. This animal model makes it possible to carefully define the distribution, dose and changing dose rate for each individual and to relate these dosimetric parameters to the biological changes observed. Extensive summaries of the experimental designs and results of the dog studies were published in two books [21,22]. Lifetime dog studies conducted at several different laboratories, were carefully integrated and monitored and were designed to determine the dose and dose-rate effects of radiation. Of special note is that the dog was used to study the impact of internally deposited radioactive materials (both high and low-LET) on cancer frequency and distribution. It was possible to relate the dose distribution with the cancer distribution so that it was possible to determine if the cancers were induced in the organs where the nuclide was concentrated. The studies demonstrated that the organ with the highest concentration and the highest dose was the organ at highest risk. With this non-uniform dose distribution very high doses could be delivered to these organs and the animals followed over their life time. After very high radiation doses were delivered at a low-dose rate, a very high (almost 100%) of the dogs developed cancer [74]. Many of the high-dose dogs had tumors in the tissue that received the highest doses, in these tissues cellular disorganization and chronic inflammatory disease were both observed. Both play a major role in the production of cancer [75]. It was possible to fit a linear dose response to each of the tissues and risk coefficients. The major problem associated with the analysis of this data was that for many organs the dose rate provided a better relationship between exposure and cancer frequency than total dose [76]. These data suggest that the use of the LNTH is not valid for these studies.

2.4. Whole body exposures

Early in the dog research projects there was a lack of careful evaluation of the influence of low doses delivered at either a high or low-dose rate. The first question addressed was what is the influence of dose rate following whole body exposures? Uniform dose distribution was achieved by exposure of dogs in a confined space to the gamma rays from an external ^{60} Co source. The details of this protocol have been published. Briefly, the dogs were exposed to whole body for 20 h per day for different time periods. Some of them were exposed for most of their lives to graded-dose rates. This made it possible to define the role of dose and dose rate on radiation induced disease. The results of these studies have been carefully summarized. Dogs were exposed to a range of well-defined dose rates and the biological changes determined [77–80]. Because of the short latent period, the primary cancer type and biological change observed in these animals was related to blood diseases. As the dose rate decreased to below about 5 rads/day (50 mGy/day) there was little change in life span. The incidence of leukemia and other blood related diseases increased at high-dose rates but was not increased when the dose rate was below this 5 rads/day (50 mGy/day) where there was no cancer frequency change. Although the sample size is small, the high dose and dose rates used suggest a threshold dose and dose rate below which no adverse biological effect can be detected in this experimental model.

2.5. Internally deposited radioactive material in dogs

2.5.1. Bone

Deposition of radioactive material in the body results in a chronic low-dose rate radiation exposure to the target organ associated with the radionuclide. Deposition of radioactive material in the bone has long been known to cause bone cancer. This was first seen in the radium dial painters who ingested large amounts of radium when they dipped their brushes in radium paint and tipped them with their mouth. The details of these studies have been carefully reviewed [81] and it was demonstrated that only dial painters with large doses to the bone had an increase in bone cancer [127]. There was an apparent threshold dose of almost 1000 rads (10 Gy) to the bone below which no cancers were observed. These studies demonstrated that the bone is a very radiation resistant organ, resulted in an appropriate tissue weighting factor for bone, and suggested a threshold in the dose-response relationship which does not support the LNTH.

Studies were initiated to determine if similar dose-response relationships would be observed following deposition of low LET beta-gamma emitting radionuclides. To study the impact of low LET radiation on bone cancer, animals were fed ^{90}Sr from before birth throughout their lives and the frequency of bone cancer determined. This radionuclide concentrates in bone and follows the same metabolic pathway as Calcium so the dose distribution in the bone was fairly uniform. These studies demonstrated that cancers were produced primarily in the bone, the site of the major dose [82]. It was determined that the frequency of bone cancers changed as a function of dose-rate, not total dose and the radiation related disease described by a simple model dependent on two variables for both high and low LET radiation [83]. Three dimensional plots of the data demonstrated that following

low-dose rate exposure that the dose-rate response was very non-linear [84]. Data analysis indicated that there was no increase in bone cancer over a very large range of radiation doses 2000 rads (20 Gy) and for induced leukemia and other soft tissue carcinomas about 1000 rads (10 Gy) [76]. For this organ there was a threshold dose and dose rate below which no differences could be detected between the controls and the exposed animals. In fact, the frequency of bone cancer was higher in the controlled animals than observed in the low dose and dose-rate groups. The author suggested that at these low-dose rates the lifespan is the limiting factor, as the dose accumulated over the lifespan of the animals is not adequate to induced cancer or life shortening. This paper provides a useful review of the results of the studies. The analysis was expanded to include animals that inhaled radioactive materials and had doses to the lung. Again the response changed as a function of dose rate not total dose [85]. Such studies demonstrate that very large total doses and dose rates are required to increase cancer frequency in the bone. Such data have been important in setting tissue weighting factors with bone being very radiation resistant [86,87]. The use of tissue weighting factors could be considered as a recognition of the thresholds demonstrated in the bone following exposure to both high and low LET delivered over a long period of time.

2.5.2. Lung

Inhaled radioactive materials concentrate in the lung and associated lymph nodes and provide the primary target for the radiation dose. For example, it was determined that when beta-gamma emitting radionuclides (^{90}Y, ^{91}Y, ^{144}Ce and ^{90}Sr) were locked into fused clay particles, the material was concentrated and retained for long periods of time in the lung and associated lymph nodes with almost no dose to the remainder of the body. These radionuclides have a wide range of physical half-lives so they deliver their dose with a changing dose-rate over a wide range of different times. Table 1 below shows the physical half-life, the effective half-life and the time required to deliver 90 percent of the total dose for each of the radionuclides [74].

The dose, dose rate, time of death, and the onset and type of cancers induced following these exposures has been previously reported [16]. When the dose and dose rates were very high the dogs died from lung disease, radiation induced pneumonitis and fibrosis in less than two years. The higher the initial dose rate from ^{90}Y, where 90 percent of the dose was delivered in eight days resulted in the earliest deaths. As the dose rate decreased the very high doses still resulted in early deaths. The evaluated lung data fits to the same functions as used in the bone and suggested that these data could also be described with similar simple functions). The dose rate to the lungs of these dogs was calculated using two different methods. First, the total dose to the lungs was divided by the time of death and used as a measure of dose rate [85]. This provided a method to convert all the data to “dose rate” and to fit all the data to very simple functions. This technique seemed to be useful in risk assessment and showed that dose rate was the important parameter for estimating cancer risk from internally deposited radioactive material. However, because of the very different effective half-lives shown above this metric does not represent the way that the energy was delivered or the biology of the response from these very different dose patterns, with ^{90}Y depositing half of its energy in 2.5 days and ^{90}Sr exposing and depositing energy for 600 days. This method of calculating dose rate is the total dose divided by the latent period of the cancer which is longer when the dose rate is delivered at a lower rate. Using this metric of dose rate ^{90}Sr was the least effective of the radionuclides and ^{90}Y the most effective per unit of dose rate.

Additional studies were conducted to determine a better metric for measuring dose rate for internally deposited radioactive material since the dose rate can change rapidly as a function of time depending on the radionuclide under study. It seemed appropriate to use the dose rate delivered at the time of the effective half-life. At this time half the dose would be delivered at a higher dose rate and half at a lower dose rate [88]. Thus, the dose rate was calculated at the point where 50 percent of the cumulative dose had been delivered (DR_{50}). Using this metric, which reflects the effective half-life of the radionuclide, it was shown that the order of effectiveness for the induction of lung cancer for the radionuclides studies was opposite than derived by Ref. [85]. That is per unit dose rate ^{90} Sr was the most effective and ^{90} Y the least. This seems to match the biology of the dose delivered per cell cycle or the damage that could be essential in the production of lung cancer.

Table 1
The physical and effective half-lives and the length of time required for deposition of 90% of the total dose for radionuclide infused aluminosilicate particles. This table is designed to illustrate the different exposure patterns following inhalation of beta gamma emitting radionuclides with a range (^{90}Sr 29 years and ^{90}Y 2.6 days) of different physical half-lives. This exposure results in a wide range of dose rate patterns that must be defined with useful metrics.

Radio nuclide	Physical half-life	Effective half-life in lung (d)	Time to deliver 90% of total dose
^{90}Sr	29 y	600	5.5 y
^{144}Ce	285 d	175	1.6 y
^{91}Y	59 d	50	0.5 y
^{90}Y	2.6 d	2.5	8.0 d

This metric provided a basis to determine what the biological impact of the dose rate would be in terms of how much dose was delivered for each cell turnover in the lung [75].) With this analysis it was possible to show that the tissue response and the induction of chronic inflammatory disease in the lung is an important biological change required for the induction of lung cancer. At very high doses per cell turnover cell killing was so extensive that the dogs died of acute lung disease. The stronger dogs that received high-dose exposure per cell turnover but survived the acute radiation syndrome of lung disease, developed a very high frequency of lung cancer regardless of the radionuclide inhaled. As the dose per cell turnover decreased to a level where these chronic inflammatory and fibrotic responses were not initiated. The lung cancer frequency drops to a level that was no higher than that observed in the control animals and the life span was not significantly reduced. When either total dose or dose per cell cycle was used as the matrix of exposure there was no increase in lung cancer frequency or life span in dogs that had a total dose of less than 2500 rads (25 Gy) to the lungs [16] or a dose per cell cycle of equal to or less than 250 rads/cell turnover (2.5 Gy/cell turnover) for ^{90}Sr, 1000 rads/cell tuirnover (10 Gy/cell turnover) for ^{144}Ce, 1100 rads/cell turnover (11 Gy/cell turnover) for ^{91}Y and 6000 rads/cell turnover (60 Gy/cell turnover) for ^{90}Y. These very high doses which did not increase cancer frequency or shorten life span make a very strong argument for a threshold below which little damage can be detected. If this high dose were to be delivered as a single whole-body acute exposure it would result in early lethality of 100 percent of the dogs. Thus, even protracting the dose over a few days and having a non-uniform distribution of the dose in the body allows for recovery that is significant in extending the life span and decreasing the cancer frequency and must be considered in modeling risk. Thus, there is a huge influence of dose rate and dose distribution on both cancer incidence and survival with a suggestion that in many cases negative terms are needed in risk evaluation [89]. Such an observation suggests different mechanism of radiation induced cancer from internally deposited radioactive material where the organ response is critical. For internally deposited radioactive materials the dose rate is low and the distribution of dose is non-uniform. This non-uniform dose distribution leaves many protective systems intact which are impacted by acute whole-body radiation exposure. For example, much of the immune system and the bone marrow is not modified by deposition of radionuclides in the lung. It seems that cancer is more of a complex tissue response and is not dependent on a single mutation or change in a single cell to modify all the key events in the critical pathways to cancer. As has been stated in the past “it takes a tissue to make a tumor (Bracellow-Hoff 2001)” and the whole tissue

and animal responses are the critical target for cancer. . It is important to be able to relate the induction of cancer in the lung to radiation induced tissue disruption, fibrosis and the induction of an inflammatory response. Without these tissue changes it seems that there is little risk for radiation induced lung cancer. Such studies point to the complex nature of cancer and suggest that all systems of the body are involved in both the induction and the protection against the production of cancer [26]. For low-dose rate exposure scenarios; tissue and whole-body responses seem to play a major role in the risk for cancer [26,90,91]. For many of these responses there are thresholds with total doses, dose per cell turnover and dose rates below which no change in risk can be observed. These thresholds demonstrate that the LNTH is not an accurate scientific evaluation of risk in the low dose region.

2.5.3. Liver

Some radionuclides concentrate in the liver, are retained for long periods of time and result in high doses to this organ. For example, ^{144}Ce- ^{144} Pr a beta gamma emitter, and several alpha emitters, ^{239} Pu, ^{241}Am, ^{252} Cf concentrate in human liver [86,87] as well as in dogs (Stannard 1987; NCRP 135), Primates [92], Grasshopper mouse [130] and the Chinese hamster [93]. Most laboratory rats and mice clear many of these radionuclides rapidly from the liver making them of little use in study of liver cancer from internally deposited radionuclides [93]. In addition, colloidal materials such as Thorotrast used for imaging, concentrated in the liver. Thorotrast is an alpha emitter and was injected into people as a contrast medium to evaluate wounds. This resulted in large alpha doses to the liver and increased human cancer incidence in the liver (NCRP 135). This material provides a good reference for the animal studies on the induction of liver cancer and derivation of risk coefficients for liver cancer (NCRP 135). Risk coefficients were derived for the liver using the LNTH model and are reported in (NCRP 135; [94,95]. From this report values of 15–40 liver cancers 10^{-4} Gy $^{-1}$ for beta-gamma emitting radionuclides and 560 10^{-4} Gy $^{-1}$ liver cancers for alpha emitters were estimated. Thus, alpha particles are about ten times as effective as beta-gamma exposures in producing cancer in the liver. The major problem and area of future research is the shape of the dose-response relationship in the low-dose region. Because of the long latent period for the induction of liver cancer (20–30 years in the low-dose groups) and the limited numbers of humans in these low-dose groups it was not possible to determine the shape of the dose-response relationship. There was a suggestion that the risk was lower in the low-dose groups suggesting non-linear dose-response relationships (ICRP 135). The role that liver injury plays in the induction of cancer in the liver is important. These very large doses produced by Thorotrast produce extensive chromosome damage and cell killing [96]. The interaction between liver damage from alcohol consumption and radiation exposure to ^{241}Am causes a marked increase in liver cancer in dogs [130]. Stimulation of cell proliferation following injection with ^{144}Ce-^{144}Pr also increases the frequency of liver cancer [97]. All these effects suggest that injury, cell proliferation, tissue disorganization and inflammatory disease have marked influence on cancer induced by low-dose rate radiation exposure. At doses below the levels required to produce these tissue effects there seems to be a threshold which provides data to suggest that the LNTH model does not apply to internally deposited radioactive material and large threshold values must be considered.

2.6. Human experience

2.6.1. High background areas

When one thinks about exposure to low-dose rate over a long time period the first thing that comes to mind is the wide range of doses from natural background. These doses vary over a wide range with some areas having background doses a couple of orders of magnitude higher than that seen in the rest of the world [98]. This range of high natural radiation areas (HNRA) are related to elevation and changes in content of natural radioactive materials in the earth like uranium and radon. A useful chart has been prepared by Dr. Noelle Metting from the DOE Low Dose Radiation Research Program (http://wwwlowdose.energy.gov and illustrates the range of natural background levels. In the U. S. 2–4 mGy/year covers the range of background dose without including medical exposures. Around the world there are areas with normal high background radiation driven by elevation and the presence of naturally occurring radionuclides. The Kerala Coast of India has a range from 8 to 20 mSv/year, Guarapari Brazil 30–40 mSv/year, and Ramsar, Iran 150–400 mSv/year.

Several epidemiological studies in the high background areas have failed to show a significant increase in cancer frequency in these areas. These studies have been reviewed and there seems to be major problems in the dosimetry associated with the studies and further research is required [99]. The fact that the dose cannot be related to the individual with the disease limits the power of the studies. However, the lack of a detectable response to these increased levels of low-dose rate exposure over a life time suggest that such low doses have minimal impact on cancer risk.

2.6.2. Added radiation dose from nuclear weapons testing

The second area of concern is addition of radiation exposure to the population above the normal existing natural background and the potential impact of these added doses which may correlate to an increase in cancer frequency. During the development of the Atomic weapons there have been huge populations, almost all the world, exposed to added low doses of radiation by fallout from nuclear tests. More than one hundred nuclear weapons were tested above ground at the Nevada test site with many more tested around the world. The total number of nuclear tests, the megatonnage yield and the country testing the weapons is shown in Table 2. The table shows that the U. S. tested the most nuclear weapons 1032 and the Soviet Union had the highest megatonnage yield 247. Thus, there was a total of 2029 weapons tested above ground with a total yield of 428 megatons. The megatonnage yield is directly related to the amount of radiation produced by each weapon. However, other variables are important in evaluating the dose, such as the location of the test relative to human populations, the elevation where the test was detonated (high elevation shots do not produce the same level of radioactive fallout as ground shots) and the composition of the weapon. Since the weapons were tested in both the northern and southern hemisphere the nuclear weapons tests resulted in an increase in background radiation dose to most of the population in the world. Areas close to the test sites received much higher doses than world-wide averages. With this increased radiation exposure from nuclear weapons tests it was postulated that there may be a detectable increase in cancer frequency. To test this hypothesis, the frequency of childhood leukemia, the cancer which is the most sensitive to radiation induced increase, was followed as a function of time in ten areas around the world. The results of these studies are shown in Fig. 4 [100].

The frequency of childhood leukemia is plotted as a function of time. The time shown on the figure was before 1950 and includes the time when testing above ground ended 1963 and followed through until 1990. Since childhood leukemia has a short latent period this time

Table 2
Nuclear tests around the world 1945–1996.

Country Testing	Number of Tests	Megatonnage Atmosphere
USA	1032	141
Soviet Union	715	247
UK	45	8
France	210	10
China	22	22
Pakistan	2	Not available
India	3	Not available
Total	2029	428

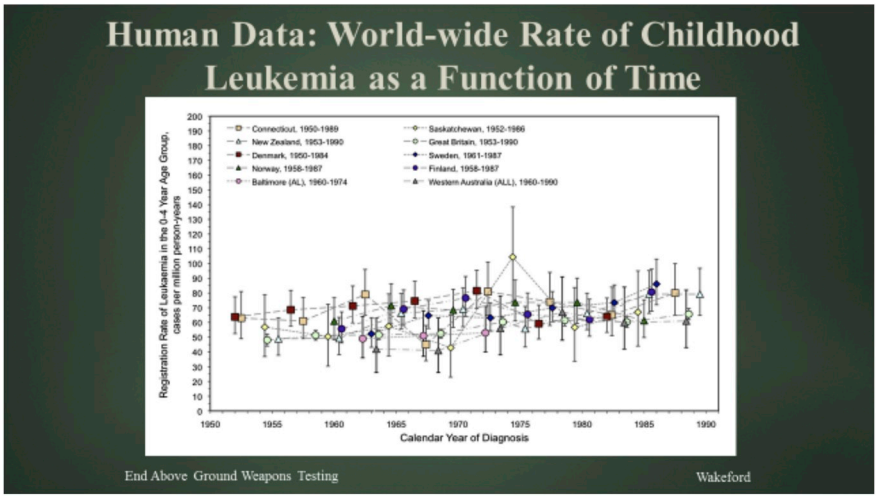

Fig. 4. The world-wide rate of childhood leukemia as a function of time. The figure illustrates that even though childhood leukemia is thought to be a cancer type that is the most sensitive to increased induction by radiation that there has been no significant change in the frequency as a function of the atomic bomb testing [100].

would be adequate to show any radiation induced increase in the disease. The figure demonstrates that there is no detectable increase in childhood leukemia as the result of nuclear weapons tests. Thus, using the most sensitive biomarker of radiation induced cancer, it was not possible to demonstrate a change in cancer as the result of world-wide fallout.

Some localized areas, like Utah, Arizona and Nevada, had population exposures from fallout that resulted in higher doses (40–60 mGy, 4.0–6.0 rad total dose) in the range of the current annual doses used to regulate nuclear exposures to workers in the nuclear industry (5 rem/year 50 mSv/year). This dose was two to three times as high as the 20 mGy/year dose used to determine that it was safe to return to the homes in Fukushima.

The distribution of radiation doses across the U. S. is shown as gamma ray exposure at 1 m above ground (Fig. 5). This figure does not take into account the total dose from beta and alpha particle exposures associated with the fallout so it may underestimate the total dose to these populations. However, these populations received their dose over a long period of time delivered at a low-dose rate.

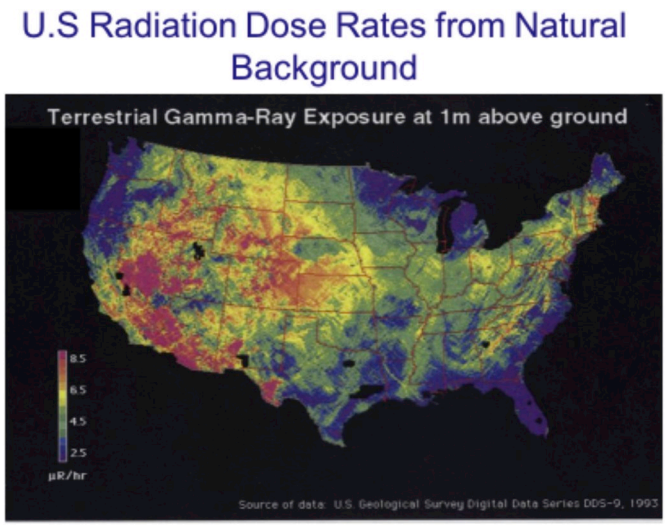

Fig. 5. This map of the U. S. shows the background dose rates for radiation measured 1 m above ground. The dose rates are shown to be influenced by both nuclear weapons testing in Nevada and elevation. This does not include exposures from internally deposited radioactive materials or beta particles.

The question then becomes, with these added low-dose rate and doses from the fallout was there an increase in cancer frequency? Data on cancer incidence in the U. S. (Fig. 6) shows that Utah has the lowest cancer frequency in the U. S. Additional data on cancer by county demonstrated that Washington County, the county with the highest fallout levels and where the highest doses occurred, have the second lowest cancer frequency in the state. To evaluate the impact of these doses on total cancer frequency in the U. S. it is of interest to compare the radiation exposures from fallout and background radiation exposures (Fig. 5) to the background cancer frequency (Fig. 6).

These figures demonstrate that the states with the highest background, the high mountain states and areas exposed to fallout from nuclear weapons testing have the lowest cancer incidence. Such data suggest that low doses of radiation delivered at a low-dose rate do not increase cancer incidence to a detectable level and that extrapolation of and predicting increased cancer risk into the low dose and dose-rate region is not supported. Thus, the LNTH is not applicable to these situations. Since these low doses are not postulated to cause a large increase in cancer any effect from the radiation could be masked by many other confounding factors such as life style and smoking. These factors have been shown to have a marked influence on the cancer incidence. Fig. 7 shows the current thinking on the environmental factors that may impact cancer frequency. What causes cancer?

Since a large fraction of the population in Utah and Idaho are members of the Church of Jesus Christ of Latter Day Saints (Mormons) and do not smoke or use alcohol, both of which are major environmental factors in the production of cancer, this life style may be the primary cause for the low-cancer frequency in these states. The urban versus rural differences and other healthier life styles may also play a role in the differences. The take home message from this discussion is that the added radiation dose from fallout delivered at a low dose did not result influence cancer frequency and is not a measurable cause of cancer. The low frequency of radiation induced cancer predicted in the low dose and dose-rate region by all the national and international committees of 5 percent/Sv or 0.005 percent/mSv is supported by these data. With a high and variable background rate of cancer about 40 percent which is dependent on sex, genetic background and life style and a high frequency of deaths produced by cancer 20 + percent it is not possible to detect any potential increase from doses in the mSv range. Radiation is not a big hitter in the production of cancer in the low-dose region. Thus, using the LNTH to predict excess cancer from fallout or natural background radiation is not supported by these documented data.

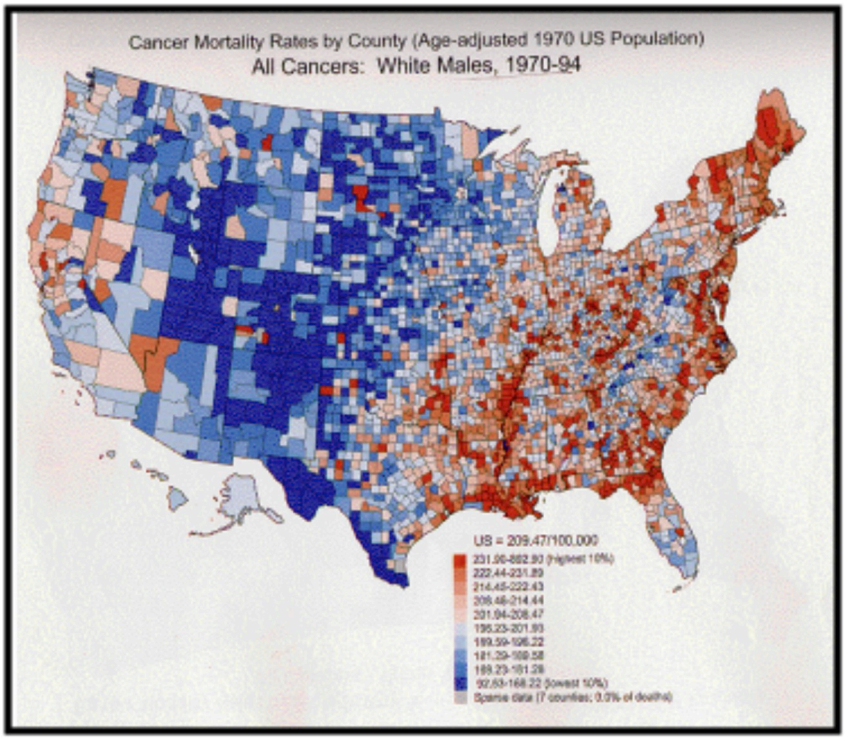

Fig. 6. This is the same map of the U. S. that illustrates the Cancer Mortality rate for white males 1970–1994. Red are high areas of cancer and blue are low. The areas with the high radiation dose rates have the lowest cancer mortality rates. A high cancer mortality rate is shown to follow the Mississippi river.

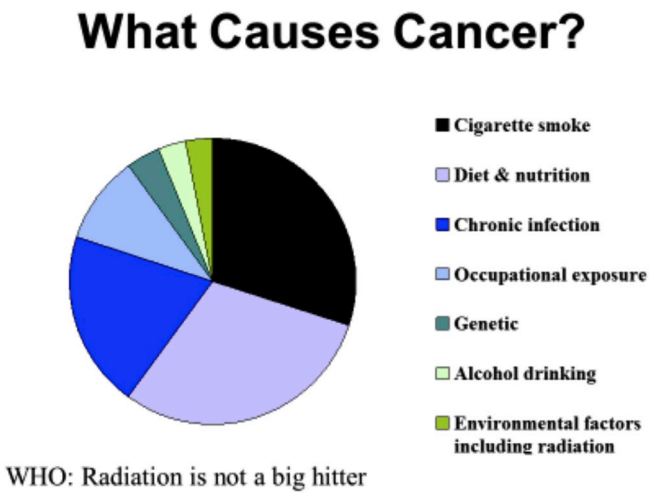

Fig. 7. This figure is taken from the World Health Organization and provides the background information needed to determine the major causes of cancer. Cigarette smoking and diet are two of the major causes of cancer. Environmental factors including radiation are one of the smaller causes of cancer. This figure illustrates how hard it is to determine the induced cancer following low dose and dose-rate radiation exposures.

3. Discussion

3.1. Paradigm shifts

With the sequencing of the genome, the development of gene expression arrays and many other technological advances in molecular biology research progressed rapidly into measuring more refined molecular and cellular endpoints. The newer data made it evident that many of the long-standing paradigm in radiation biology needed to be reconsidered [24,27]. The required paradigm shifts have forced the field of radiation biology to take a new look at many well accepted concepts in the field. For example, the hit theory for describing radiation biology needs to be replaced by a wider view of radiation biology. Since the discovery of radiation induced bystander effects, cell/cell and cell/tissue communication, result in a much larger target than a single cell for the interaction of radiation with biological systems. The single hit on a DNA molecular may produce a mutation in a single cell as an explanation for radiation induced cancer. This is not the whole story, there is a need to expand the concept to include more of a systems biology approach. DNA hits by radiation trigger many biological processes including radiation induced changes in gene and protein expression as well as post radiation modification of proteins. Research also demonstrated that radiation can induce many epigenetic changes which must be considered in risk evaluations [101]. These changes were further supported by unpredicted changes in physiology and critical pathways to cancer. It became obvious that the changes induced at the molecular level by single acute exposure to high doses were very different than those induced by low doses of radiation.

The observation of adaptive protection following low doses brought into question the long standing LNTH theory of radiation induced damage. Many molecular and cellular endpoints showed a decrease in biological response below that seen in the controls following low doses or low-dose rate radiation exposure. Each of these important paradigm shifts must be reviewed, discussed and the potential impact on radiation rules and standards evaluated.

3.2. Hit theory

The interaction of radiation with matter is described as individual energetic events interacting with single cells. These events have enough energy to cause ionizations and produce changes in important molecules. This was called the “hit theory” and many biological changes were directly related to the number of hits, the time between hits and the type of hits or energy deposition events. This provided the framework for the development of the LNTH since single hits produced important single changes in critical molecules and it was postulated that

every ionization resulted in damage and increased risk. This concept was used for most of the past research in the field of radiation biology. It was not until the development of microbeams and the ability to hit a known cell and follow its response as well as the response of neighboring cells that the hit theory was called into question. It was observed that when a cell is hit, it communicates with the neighboring cells and the total biological response is dependent not only on the cells hit but also on the response of the organ or tissue. This made the target for radiation interaction much larger than the individual cell and suggested that, much like chemicals that produce similar changes, radiation response is not a single cell event [90]. It was determined the cell/cell communication or bystander effects occurred both *in vitro* and in animal models [102,103] and *in vivo* [104].

3.3. Mutation theory

The genetic or mutation theory of cancer suggested that a single cell receives a single hit, produces a change in DNA and this would result in a radiation induced mutation. Such an altered cell could have a proliferative advantage which would, following cell proliferation, expand the mutated cell population. Further changes would result in loss of control of cell division, metaphasis and cancer. This theory was critical in the development of the LNTH for the description of risk from radiation. Recent research suggested that radiation induced cancer may also work though a wide variety of different mechanism and physiological pathways some of which may be triggered by radiation induced mutations in individual cells.

3.4. Genomic instability

The induction of genomic instability was observed in recent research resulting in the loss of genetic control and the observation of multiple genetic alterations in cell population. This condition was induced by high acute exposure to radiation. Genomic instability has been defined as a late occurring radiation induced change where the target for its induction is much larger than a gene and the cells lose genetic control. Following radiation exposure no changes are observed for several cell divisions. After multiple cell divisions the cells lose genetic control and many types of biological changes are observed, for example chromosome aberrations, polyploidy, apoptosis, and formation of clones with defined chromosome damage and multiple mutations. Genomic instability is often observed during the early stages of cancer development for many types of cancer. Genomic instability has been demonstrated both *in vitro* [105] and in animal models [106]. In all these studies the genetic background of the cells or animals played an important role in the induction of genomic instability. Multiple studies have attempted to demonstrate the induction of genomic instability in normal human cells [107] or human populations [108] and have not been able to demonstrate it. Research on radiation induced genomic instability has been reviewed [109]. Because of the lack of low dose and low-dose rate data it is not possible to estimate the impact of dose-rate on the induction of genomic instability and its potential impact on the LNTH. There have been few studies on the induction of genomic instability in the low dose and dose-rate region. Thus, there remains a controversy on the role of low dose radiation induced genomic instability and cancer induction [110]. This is an area that requires additional research. The data to date have not demonstrated genomic instability in induced by low dose or dose rate and suggest that it may not impact in these regions of the dose-response relationship.

3.5. Adaptive protection

Adaptive responses were first observed and reviewed by Wolff [111]. In their studies where cells were exposed to a small “tickle dose”, followed by a larger “challenge dose”. With this protocol it was observed that the pre-exposure to the small dose made the cells radiation resistant to the induction of chromosome aberrations. The small dose activated protective mechanisms that reduced the frequency of the aberrations below the level predicted by the sum of the two doses. This was only observed if the two doses were separated in time by a few hours. Thus, the potential impact of this adaptive response on cancer risk and radiation standards was thought to be minimal.

As research progressed it was demonstrated that a new type of adaptive response was observed. That is when a small dose of radiation was delivered at either a high or low-dose rate it produced a decrease in many key events in the critical pathways to cancer induction below the level observed in the controls [20]. This adaptive response was reviewed and defined by Feinendegen [26] as adaptive protection. This observation was first related to the induction of cell transformation, a critical step as the cells progress from normal to acquiring the characteristics needed to develop cancer. Many studies were conducted to measure radiation induced cell transformation and demonstrated that low doses of ionizing radiation delivered at either a high [112]; [62]) or low-dose rate [65] decreased the spontaneous frequency of cell transformation below that observed in control cells receiving no radiation exposure. If cell transformation *in vitro* represents a key event in the pathway as cells progress toward radiation induced cancer, then such cellular studies suggest that a negative or protective value may be required in risk models [89,113] which would directly attack the LNTH model. Other endpoints such as the induction of mutations [64,114] also demonstrated a decrease in the frequency of mutations by small doses of radiation.

3.6. Selective apoptosis

The induction of selective apoptosis, programmed cell death, was demonstrated [61]. In these studies, small doses of radiation resulted in selective killing of transformed cells which would result in a decrease in potential cancer cells below the level without the radiation exposure. This observation would provide a mechanism for the observed decrease in cell transformation and mutations described above.

3.7. Whole animal and tissue responses

3.7.1. Reactive oxygen status and inflammatory disease

The induction of chronic inflammatory disease in any tissue can result in an increase in the risk for cancer in that tissue or organ. Tissue damage was evaluated in chemical studies and it was determined that high dose chemical carcinogens which produced extensive tissue damage in the target organs were for the most part, not responsible for the induction of cancers [115]. As the mechanisms of carcinogenesis have been further studied it has become evident that radiation induced increases in levels of reactive oxygen species (ROS) in the tissues and chronic inflammatory disease play an important role in cancer induction. This has been demonstrated for a number of different tissues, bone, lung and liver discussed above [75,76,85,116]. The induction of anti-oxidant, anti-inflammatory cytokines down-regulate these reactive species and restore homeostasis (Schaue et al., 2012). Many molecular and cellular responses have been measured as a function of dose and dose-rate. Changes in gene expression with the up-regulation of genes involved in anti-inflammatory disease have been demonstrated [45,48,117]. These changes seem to be related to changes in mitochondria [118] and the ROS status of the cells [119]: [120]. Low doses of radiation decrease the levels of reactive oxygen species in the tissue which suggest protection against cancer. Other studies have demonstrated that radio-protective chemicals can have a similar impact on the ROS status of the tissues [121]. Low dose and dose-rate radiation can also induce modification of genes can alter ROS status by changing SH-containing chemicals by alteration of MnSOD and SOD-2 [46,122]. These changes have also been postulated to decrease cancer risk. Low

doses can also modify metabolic pathways that may influence the induction of cancer [123]. The data suggests that the mechanisms of action for low doses and high doses of radiation are different and that low doses may result in a decrease in cancer risk. These data help demonstrate that the mechanisms of action are different at high and low doses and do not support the LNTH.

4. Conclusions

By reviewing the literature at all levels of biological organization from the molecular to humans several important points are noted.

- At the cell and molecular level it is obvious that the responses to low doses and dose rates are very different from those following acute high doses. This suggests different mechanisms of action and different metabolic pathways are activated by high and low doses of radiation. Such data provides a strong basis for needed paradigm shifts in radiation biology. These paradigm shifts do not support the scientific basis for the LNTH.
- At the animal level, there are large data bases that demonstrate marked thresholds in the dose-response relationship for cancer induction. This is especially true for non-uniformly distributed internally deposited radioactive materials that can deliver very high doses at low-dose rates. These thresholds have been demonstrated in bone, liver and lung and does not support the LNTH.
- Human data on doses and dose rates, near or a few orders of magnitude above natural background, show no measurable change in cancer frequency. Such data demonstrates that the cancer risk values currently used are conservative and do not underestimate risk. Because of the low incidence of radiation induced cancer per mSv or mGy exposure in humans study populations have to be very large to detect changes predicted by the LNTH. Currently in the range of natural background radiation doses and dose-rates changes in cancer frequency have not been detected.

The LNTH has been useful in setting regulations and has been useful in worker protection in the past. However, extensive past and present research has demonstrated that LNTH is not a good scientific representation of the responses to radiation in the low dose and dose-rate region and should not be used in combination with collective dose to predict cancer frequency. The over-estimate of cancer risk using the LNTH has resulted in extremely high costs with no medical benefit. In addition, the suggestion that every ionization increases risk has contributed to many practices and rules that result in huge expenses and public fear [124]. This excessive fear has caused harm in the past. For example, in Japan during the Fukushima event, the measured doses were not projected to increase cancer frequency, fear and policy resulted in evacuation which resulted in the death of many people. Fear has driven public perception in many areas and made it difficult to use radiation in many areas (medicine, agriculture and power) where it has great benefit. The present manuscript provides an overview of the science associated with radiation at all levels of biological organization from the molecular to humans and demonstrates the need for serious paradigm shifts in the field of radiation biology and suggests the need to reconsider the use of the LNTH in rule making and regulations.

Declarations of interest

ALB was partially funded for this research by Bruce Power. He does not view this as a conflict of interest since they had no input into the production of this manuscript. ALB worked for twenty years at the Lovelace ITRI but was not directly involved in conducting the dog studies. He reviewed and provided early data input.

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Transparency document

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References

[1] D.L. Preston, E. Ron, S. Tokuoka, S. Funamoto, N. Nishi, M. Soda, K. Mabuchi, K. Komada, Solid cancer incidence in atomic bomb survivors: 1958–1998, Radiat. Res. 168 (2007) 1–64.
[2] W.-L. Hsu, D.L. Preston, M. Soda, H. Sugiyama, S. Funomoto, K. Kodama, A. Kimura, N. Kamada, H. Dohy, M. Tomonaga, M. Iwanaga, Y. Miyazaki, H.M. Cullings, A. Suyama, K. Ozasa, R.E. Shore, K. Mabuchi, The incidence of leukemia lymphoma and multiple myeloma among atomic bomb survivors, Radiat. Res. 179 (2013) 361–382.
[3] K. Ozasa, Y. Shimizu, A. Suyama, F. Kasagi, M. Soda, E.J. Grant, R. Sakata, H. Sugiyama, K. Kodama, Studies of the mortality of atomic bomb survivors, Report 14, 1950–2003: an overview of cancer and non-cancer diseases, Radiat. Res. 177 (2012) 27–39.
[4] M.P. Little, E.J. Tawn, I. Tzoulaki, R. Wakeford, G. Hildebrandt, F. Paris, S. Tapio, P. Elliott, A systematic review of epidemiological associations between low and moderate doses of ionizing radiation and late cardiovascular effects, and their possible mechanisms, Radiat. Res. 169 (2008) 99–109.
[5] E.A. Blakely, Lauriston S. Taylor Lecture on radiation protection and measurements: what makes particle radiation so effective? Health Phys. 103 (2012) 508–528.
[6] Y. Shimizu, K. Kodama, N. Nishi, F. Kasagi, A. Sutyama, M. Soda, E.J. Grant, H. Sugiyama, R. Sakata, H. Moriwaki, M. Hayashi, M. Konda, R.E. Shore, Radiation exposure and circulatory disease risk, BMJ 340 (2010) b5349.
[7] UNSCEAR, United Nations Scientific Committee on the Effects of Atomic Radiation UNSCEAR 2006 Report. Annex B. Epidemiological Evaluation of Cardiovascular Disease and Other Non-cancer Disease Following Radiation Exposure, United Nations, New York, 2008, pp. 325–383.
[8] National Council for Radiation Protection and Measurements (NCRP), Influence of Dose and its Distribution in Time on Dose-response Relationships for Low LET Radiation vol. 64, NCRP Report No, Bethesda, MD, 1980.
[9] National Council for Radiation Protection and Measurements (NCRP), Limitation of Exposure to Ionizing Radiation vol. 116, NCRP Report No, Bethesda, MD, 1993.
[10] UNSCEAR, United Nations Scientific Committee on the Effects of Atomic Radiation United Nations Scientific Committee on the Effects of Atomic Radiation. Annex F: Influence of Dose and Dose-rate on Stochastic Effects of Radiation, United Nations, New York, 1993, pp. 620–728.
[11] UNSCEAR, United Nations Scientific Committee on the Effects of Atomic Radiation. Biological Mechanisms of Radiation Actions at Low Doses. A White Paper to Guide the Scientific Committee's Future Programme of Work, United Nations, New York, 2012, pp. 1–35.
[12] National Research Council/National Academy of Sciences (NRC/NAS), Health Risks from Exposure to Low Levels of Ionizing Radiation (BEIR VII Phase 2). Committee to Assess Health Risks from Exposure to Low Levels of Ionizing Radiation, National Academy Press, Washington, DC, 2006.
[13] M. Tubiana, A. Aurengo, D. Averbeck, A. Bonnin, B. Le Guen, R. Masse, R. Monier, A.J. Valleron, F. de Vathaire, Dose-effect Relationships and Estimation of the Carcinogenic Effects of Low Doses of Ionization Radiation, Academie National de Medicine, Institute de France Academie des Sciences, Paris, 2005 ([English translation]).
[14] Health Physics Society Position Statement, Radiation Risk in Perspective: Position Statement of the Health Physics Society, (2016) PSO10-3.
[15] D.J. Brenner, C.D. Elliston, Estimated radiation risks potentially associated with full-body CT screening, Radiology 232 (2004) 735–738.
[16] A.L. Brooks, P.E. Eberline, L.A. Couch, B.B. Boecker, The role of Dose-Rate on Risk from internally-deposited radionuclides and the potential need to separate dose-rate effectiveness factor (DREF) from the dose and dose-rate effectiveness factor (DDREF), Health Phys. 97 (5) (2009) 458–469.
[17] W. Ruhm, G.E. Woloschak, R.E. Shore, T.V. Azizova, B. Grosche, O. Niwa, S. Akiba, T. Ono, K. Suzuki, T. Iwasaki, N. Ban, M. Kai, C.H. Clement, S. Bouffler, H. Toma, N. Hamada, Dose and dose-rate effects on ionizing radiation: a discussion in the light of radiological protection, Radiat. Environ. Biophys. (2015), https://doi.org/10.1007/s00411-015-0613-6.
[18] D.G. Hoel, Comments on the DDREF estimate of the BEIR VII committee, Health Phys. 108 (2015) 351–356.
[19] B.M. Haley, T. Panesku, D.J. Grdina, G.E. Woloschak, The increase in animal mortality risk following exposure to sparsely ionizing radiation is not linear

quadratic with dose, PLoS One 10 (12) (2015) e0140989, https://doi.org/10.1371/journal.pone.0140989.
[20] A.L. Brooks, D.G. Hoel, R.J. Preston, The Role of Dose Rate in Radiation Cancer Risk: evaluating the effect of dose rate at the molecular, cellular and tissue levels using key events in critical pathways following exposure to low LET radiation, Int. J. Radiat. Biol. 92 (8) (2016) 405–426.
[21] J.N. Stannard, R.W. Baalman (Ed.), Radioactivity and Health: a History, 1988 DOE/RL/01830-T59 (DE88013791) Distribution Category UC-408.
[22] R.C. Thompson, Life-span Effects of Ionizing Radiation in the Beagle Dog. PNL 6822, UC-408, (1989).
[23] A.L. Brooks, A commentary on: "A history of the United States department of energy (DOE) low dose radiation research program: 1998-2008, Radiat. Res. 831 (4) (2015) 375–381.
[24] A.L. Brooks, Paradigm shifts in radiation biology: their impact on intervention for radiation induced disease, Radiat. Res. 164 (2005) 454–461.
[25] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646–674.
[26] L.E. Feinendegen, M. Polycove, R.D. Neumann, Whole-body responses to low-level radiation exposure: new concepts in mammalian radiobiology, Exp. Hematol. (Oak Ridge) 35 (2007) 37–46.
[27] A.L. Brooks, Low Dose Radiation: the History of the, U.S. Department of Energy Research Program. Washington State University Press. Pullman, Washington, 2018.
[28] Y. Adeleye, M. Andersen, R. Clewell, M. Davies, M. Dent, S. Edwards, P. Fowler, S. Malcomber, B. Nicol, A. Scott, S. Scott, B. Sun, C. Westmoreland, A. White, O. Zhang, P.L. Charmichael, Implementing toxicity testing in the 21st century (TT21C): making safety decisions using toxicity pathways and progress in a prototype risk assessment, Toxicology 332 (2015) 102–111.
[29] S.W. Edwards, Y.-M. Tan, D.L. Villeneuve, M.E. Meek, C.A. McQueen, Adverse outcome pathways–organizing toxicological information to improve decision making, J. Pharmacol. Exp. Therapeut. 356 (2016) 170–181.
[30] R.J. Preston, Integrating basic radiobiological science and epidemiological studies: why and how, Health Phys. 108 (2015) 125–130.
[31] B.R. Scott, Low-dose fallacy associated with the linear-no-threshold model, Hum. Exp. Toxicol. 27 (2008) 163–168.
[32] B.R. Scott, C.L. Sanders, R.E. Mitchel, D.R. Boreham, CT scans may reduce rather than increase the risk of cancer, J. Am. Phys. Surg. 13 (2008) 9–11.
[33] W. Olipitz, D. Wiktor-Brown, J. Shuga, B. Pang, J. McFaline, P. Lonkar, A. Thomas, J.T. Mutamba, J.S. Greenberger, L.D. Sampson, P.C. Dedon, J.C. Yanch, B.P. Engelward, Integrated molecular analysis indicates undetectable DNA damage in mice after continuous irradiation at 400-fold natural background radiation, Environ. Health Perspect. 120 (2012) 1130–1135.
[34] T. Neumaier, J. Swenson, C. Pham, A. Plyzos, A.T. Lo, P. Yang, J. Dyball, A. Asithamby, D.J. Chen, M.J. Bissell, S. Thalhammer, S.V. Cotes, Evidence for formation of DNA repair centers and dose-response nonlinearity in human cells, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 443–448.
[35] S. Grudzenski, A. Raths, S. Conrad, C.E. Rube, M. L€obrich, Inducible response required for repair of low-dose radiation damage in human fibroblasts, Proc. Natl. Acad. Sci. Unit. States Am. 12 (2010) 14205–14210.
[36] K. Rothkamm, M. Lobrich, Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray dose, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 5057–5062.
[37] J.K. Wickliffe, B.E. Rogers, R.K. Chesser, C.J. Phillips, S.P. Gaschak, R.J. Baker, Mitochondrial DNA heteroplasmy in laboratory mice experimentally enclosed in the radioactive Chernobyl environment, Radiat. Res. 159 (2002) 458–464.
[38] B.E. Rogers, R.K. Chesser, J.K. Wickliffe, C.J. Phillips, R.J. Baker, Subchronic exposure of BALB/C and C57BL/6 strains of Mus musculus to the radioactive environment of the Chernobyl, Ukraine exclusion zone, Environ. Toxicol. Chem. 20 (2001) 2830–2835.
[39] J.A. Heddle, M.C. Cimino, M. Hayashi, F. Romagna, M.D. Shelby, J.D. Tucker, P. Vanparys, J.T. McGregor, Micronuclei as an index of cytogenetic damage: past, present and future, Environ. Mol. Mutagen. 18 (1991) 277–291.
[40] M.A. Coleman, A.J. Wyrobek, Differential transcript modulation of genes after low vs. high doses of ionizing radiation, in: A.B. Wolbarst, R.G. Samenhof, W.H. Hendee (Eds.), Advances in Medical Physics, Medical Physics Publishing, Madison, WI, 2006, pp. 273–276.
[41] A.L. Fachin, S.S. Mello, P. Sandrin-Garcia, C.M. Junta, T. Ghilardi-Netto, E.A. Danadi, S. Passos Gad, E.T. Sakamoto-Hojo, Gene expression profiles in radiation workers occupationally exposed to ionizing radiation, J. Radiat. Res. 50 (2009) 61–71.
[42] E. Yin, D.O. Nelson, M.A. Coleman, L.E. Peterson, A.J. Wyrobek, Gene expression changes in mouse brain after exposure to low-dose ionizing radiation, Int. J. Radiat. Biol. 79 (2003) 759–775.
[44] S.M. De Toledo, N. Asaad, P. Venkatachalam, L. Li, R.W. Howell, D.R. Spitz, E.I. Azzam, Adaptive responses to low-dose/low-dose-rate gamma rays in normal human fibroblasts: the role of growth architecture and oxidative metabolism, Radiat. Res. 166 (2006) 849–857.
[45] M. Fan, K.M. Ahmed, M.C. Coleman, D.R. Spitz, J.J. Li, Nuclear factor k (kappa b) and manganese superoxide dismutase mediate adaptive radioresistance in low-dose irradiated mouse skin epithelial cells, Cancer Res. 67 (2007) 3220–3228.
[46] G. Guo, Y. Yan-Sanders, B.D. Lyn-Cook, T. Wang, D. Tamae, J. Ogi, A. Khaletskiy, Z. Li, C. Weydert, J.A. Longmate, T.T. Huang, D.R. Spitz, L.W. Oberley, J.J. Li, Manganese superoxide dismutase-mediated gene expression in radiation-induced adaptive responses, Mol. Cell Biol. 23 (2003) 2362–2378.
[47] L.T. Dauer, A.L. Brooks, D.G. Hoel, W.F. Morgan, D. Stram, P. Tran, Review and evaluation of updated research on the health effects associated with low-dose ionizing radiation, Radiat. Protect. Dosim. 140 (2010) 103–136.
[48] S.A. Amundson, R.A. Lee, C.A. Koch-Paiz, Differential responses of stress genes to low dose-rate irradiation, Mol. Canc. Res. 1 (2003) 445–452.
[49] S.A. Ghandhi, L.B. Smilenov, C.D. Elliston, M. Chowdhury, S.A. Amundson, Radiation dose-rate effects on gene expression for human biodosimetry, BMC Med. Genomics 8 (2015) 22–32.
[50] S. Paul, S.A. Amundson, Development of gene expression signatures for practical radiation biodosimetry, Int. J. Radiat. Oncol. Biol. Phys. 73 (2008) 1236–1244.
[51] S. Paul, L.B. Smilenov, C.D. Elliston, S.A. Amundson, Radiation dose-rate effects on gene expression in mouse biodosimetry model, Radiat. Res. 184 (2015) 24–32.
[52] A.L. Brooks, Chromosome damage in liver cells from low dose rate alpha, beta and gamma irradiation: derivation of RBE, Science 190 (1975) 1090–1092.
[53] R.J. Purrott, E. Reeder, The effect of changes in dose rate on the yield of chromosome aberrations in human lymphocytes exposed to gamma rays, Mutat. Res. 35 (1976) 437–444.
[54] W.A. Hsieh, W. Deng, W.P. Chang, N. Galvan, C.L. Owens, D.P. Morrison, K.L. Gale, J.N. Lucas, Alpha coefficient of dose-response for chromosome translocations measured by FISH in human lymphocytes exposed to chronic 60Co gamma rays at body temperature, Int. J. Radiat. Biol. 75 (1999) 435–439.
[55] J.N. Lucas, F. Hill, T. Fester, T. Straume, Dose-response curve for chromosome translocations measured in human lymphocytes exposed to 60Co gamma rays, Health Phys. 68 (1999) 761–765.
[56] A.L. Brooks, R.O. McClellan, Cytogenetic effects of Strontium-90 on the bone marrow of the Chinese Hamster, Nature 219 (1968) 761–763.
[57] A.L. Brooks, R.O. McClellan, Chromosome aberrations and other effects produced by ^{90}Sr-^{90}Y in Chinese hamsters, Int. J. Radiat. Biol. 16 (1969) 545–561.
[58] A.L. Brooks, R. Miick, R.L. Buschbom, M.K. Murphy, M.A. Khan, The role of dose rate in the induction of micronuclei in deep-lung fibroblasts in vivo after exposure to ^{60}Co gamma rays, Radiat. Res. 144 (1995) 114–118.
[59] J.S. Bedford, J.B. Mitchell, Dose rate effects in synchronous mammalian cells in culture, Radiat. Res. 54 (1973) 316–327.
[60] M.S. Mendonca, K.L. Howard, D.L. Farrington, L.A. Desmond, T.M. Temples, B.M. Mayhugh, J.J. Pink, D.A. Boothman, Delayed apoptotic responses associated with radiation-induced neoplastic transformation of human hybrid cells, Cancer Res. 59 (1999) 3972–3979.
[61] G. Bauer, Low dose radiation and intercellular induction of apoptosis: potential implications for the control of oncogenesis, Int. J. Radiat. Biol. 83 (2007) 873–888.
[62] J.L. Redpath, In vitro radiation-induced neoplastic transformation: suppressive effects at low doses, Radiat. Res. 167 (2007) 345–346.
[63] J.L. Redpath, Q. Lu, X. Lao, S. Molloi, E. Elmore, Low doses of diagnostic energy X-rays protect against neoplastic transformation in vitro, Int. J. Radiat. Biol. 79 (2003) 235–240.
[64] P.J. Sykes, G. Zeng, T.K. Day, A.M. Hooker, B.J. Blyth, M. Bhat, W.D. Tilley, Non-linear chromosomal inversion response in prostate after low dose X-radiation exposure, Mutat. Res. 602 (2006) 65–73.
[65] E. Elmore, X.Y. Lao, R. Kapadia, J.L. Redpath, The effect of dose rate on radiation-induced neoplastic transformation in vitro by low doses of Low-LET radiation, Radiat. Res. 166 (2006) 832–838.
[66] W.L. Russell, L.B. Russell, M.B. Cupp, Dependence of mutation frequency on radiation dose-rate in female mice, Proc. Natl. Acad. Sci. U.S.A. 45 (1959) 18–23.
[67] W.L. Russell, L.B. Russell, E.M. Kelly, Radiation dose rate and mutation frequency, Science 128 (1958) 1546–1550.
[68] L.B. Russell, P.R. Hunsicker, The effect of dose rate on the frequency of specific-locus mutations in mouse spermatogonia is restricted to larger lesions: a retrospective analysis of historical data, Radiat. Res. 177 (2012) 555–564.
[69] R.L. Ullrich, J.B. Storer, Influence of gamma irradiation on the development of neoplastic disease in mice. 3. Dose-rate effects, Radiat. Res. 80 (1979) 325–342.
[70] S.A. Benjamin, A.C. Lee, G.M. Angleton, W.J. Saunders, T.J. Keefe, C.H. Mallinckrodt, Mortality in Beagles irradiated during prenatal and postnatal development. 2. Contribution of benign and malignant neoplasia, Radiat. Res. 150 (1998) 330–348.
[71] N.A. Gillett, B.A. Muggenburg, B.B. Boecker, F.F. Hahn, F.A. Seiler, A.H. Rebar, R.K. Jones, R.O. McClellan, Single inhalation exposure to $^{90}SrCl_2$ in the beagle dog: hematological effects, Radiat. Res. 110 (1987) 267–288.
[72] R.C. Pfleger, B.B. Boecker, H.C. Redman, J.A. Pickrell, J.L. Mauderly, R.K. Jones, S.A. Benjamin, R.O. McClellan, Biological alterations resulting from chronic lung irradiation. 1. The pulmonary lipid composition, physiology, and pathology after inhalation by Beagle dogs of 144-Ce labeled fused clay aerosols, Radiat. Res. 63 (1975) 275–298.
[73] F.F. Hahn, B.B. Boecker, R.G. Cuddihy, C.H. Hobbs, R.O. McClellan, M.B. Snipes, Influence of radiation dose patterns on lung tumor incidence in dogs that inhaled beta emitters: a preliminary report, Radiat. Res. 96 (1983) 505–517.
[74] ITRI Annual Report 139 Lovelace Institute Tissue Archive [Internet]. Data; Available from: http://janus.northwestern.edu/lovelace/index.php.
[75] S. Puukila, C. Thome, A.L. Brooks, G. Woloschak, D.R. Boreham, The role of radiation induced injury on lung cancer, Cancers 9 (2017) 89–101.
[76] O.G. Raabe, Concerning the health effects of internally deposited radionuclides, Health Phys. 98 (3) (2010) 515–536.
[77] B.A. Carnes, T.E. Fritz, Responses of the Beagle to protracted irradiation. 1. Effect of the total dose and dose-rate, Radiat. Res. 128 (1991) 125–132.
[78] B.A. Carnes, T.E. Fritz, Continuous irradiation of beagles with gamma rays, Radiat. Res. 136 (1993) 103–110.
[79] T.M. Seed, S.M. Cullen, L.V. Kaspar, D.V. Tolle, T.E. Fritz, Hemopathologic consequences of protracted gamma irradiation: alterations in granulocyte reserves and granulocyte mobilization, Blood 56 (1890) 42–51.

[80] W.P. Norris, T.E. Fritz, C.E. Rehfeld, C.M. Poole, The response of the Beagle dog to cobolt-60 gamma radiation: determination of the LD50(30) and description of associated changes, Radiat. Res. 35 (1968) 681–708.
[81] R.E. Rowland, Radium in Humans: a Review of U.S. Studies, Argonne National Laboratory, Argonne, IL, 1994 ANL/ER-3.
[82] O.G. Raabe, N.J. Parks, Skeletal uptake and lifetime retention of 90Sr and 226Ra in beagles, Radiat. Res. 133 (1993) 204–218.
[83] O.G. Raabe, Scaling of fatal cancer risks from laboratory animals to man, Health Phys. 57 (Suppl 1) (1989) 419–432.
[84] O.G. Raabe, Three-dimensional dose-response models of competing risks and natural life span, Fund. Appl. Toxicol. 8 (1987) 465–473.
[85] O.G. Raabe, Concerning ionizing radiation-induced cancer from internally deposited radionuclides, Health Phys. 91 (10) (2015) 810–819.
[86] ICRP, International Commission of Radiation Protection: Recommendations. Report No. 60, Pergamon Press, New York, 1991.
[87] International Commission on Radiological Protection (ICRP), Limits for Intakes of Radionuclides by Workers, Recommendations of the International Commission on Radiological Protection. ICRP Publication 30 Part 1 (Annals of the ICRP 2, Elsevier Science, New York), 1991.
[88] S. Puukila, C. Thome, A.L. Brooks, G. Wolochak, D.R. Boreham, The influence of changing dose rate patterns from inhaled beta-gamma emitting radionuclides on lung cancer, Int. J. Radiat. Biol. 94 (11) (2018) 955–966.
[89] B.R. Scott, J. Di Palma, Sparsely ionizing diagnostic and natural background radiations are likely preventing cancer and other genomic-instability-associated disease, Dose Resp. 5 (2007) 230–255.
[90] M.H. Barcellos-Hoff, It takes a tissue to make a tumor: epigenetics, cancer and the microenvironment, J. Mammary Gland Biol. Neoplasia 6 (2001) 213–221.
[91] M.H. Barcellos-Hoff, A.L. Brooks, Extra-cellular signaling through the microenvironment: a hypothesis relating carcinogenesis, bystander effects and genomic instability, Radiat. Res. 156 (2001) 618–627.
[92] P.W. Durbin, Plutonium-health implications for man. Plutonium in mammals: influence of plutonium chemistry, route of administration and physiological status of the animal on initial distribution on and long-term metabolism, Health Phys. 49 (1975) 495–510.
[93] B. Sturbaum, A.L. Brooks, R.O. McClellan, Tissue distribution and dosimetry of ^{144}Ce in Chinese hamsters, Radiat. Res. 44 (1970) 359–367.
[94] G. Van Kaick, A. Dalheimer, S. Honik, A. Kaul, D. Liebermann, H. Luhrs, A. Spiethoff, K. Wegener, H. Wesch, The German Thorotrast study: recent results and assessment of risk, Radiat. Res. 152 (1999) S64–S71.
[95] M. Andersson, H.H. Storm, Cancer incidence among Danish Thorotrast-exposed patients, J. Natl. Cancer Inst. 84 (1992) 1318–1325.
[96] R.A. Guilmette, N.A. Gillett, A.F. Edison, W.C. Griffith, A.L. Brooks, The influence of non-uniform alpha irradiation of Chinese hamster liver on chromosome damage and the induction of cancer, in: D.M. Taylor, C.H. Mays, G.B. Gerber (Eds.), Risks from Radium and Thorotrast, Thomas RG. British Institute of Radiology, London, 1989, pp. 142–148.
[97] A.L. Brooks, S.A. Benjamin, R.K. Jones, R.O. McClellan, Interaction of ^{144}Ce and partial hepatectomy in the production of liver neoplasms in the Chinese hamster, Radiat. Res. 91 (1982) 573–588.
[98] UNSCEAR, United Nations Scientific Committee on the Effects of Atomic Radiation. Sources and Effects of Ionizing Radiation. (UNSCEAR) 2000. Report to the General Assembly, with Scientific Annexes. Volume II Effects, United Nations Publications, New York, 2000.
[99] R. Shore, Need Help Finding This Publication, (2018).
[100] R. Wakeford, S.C. Darby, M.F.G. Murphy, Temporal trends in childhood leukemia incidence following exposure to radioactive fallout from atmospheric nuclear weapons testing, Radiat. Environ. Biophys. 49 (2010) 213–227.
[101] A.J. Bernal, D.C. Dolinoy, D. Huang, R.L. Jirtle, Adaptive radiation induced epigenetic alterations mitigated by antioxidants, Faseb. J. 27 (2) (2013) 665–671 2013.
[102] C.R. Geard, G. Jenkins-Baker, S.A. Marino, B. Ponnaiya, Novel approaches with track segment alpha particles and cell co-cultures in studies of bystander effects, Radiat. Protect. Dosim. 99 (2002) 233–236.
[103] O.V. Belyakov, A.M. Malcolmson, M. Folkard, K.M. Prise, B.D. Michael, Direct evidence for a bystander effect of ionizing radiation in primary human fibroblasts, Br. J. Canc. 84 (2001) 674–679.
[104] A.L. Brooks, Evidence for “bystander effects” *in vivo*, Hum. Exp. Toxicol. 23 (2004) 67–70.
[105] W.F. Morgan, J.P. Day, M.I. Kaplan, E.M. McGhee, C.L. Limoli, Genomic instability induced by ionizing radiation, Radiat. Res. 146 (1996) 247–258.
[106] B. Ponnaiya, M.N. Cornforth, R.L. Ullrich, Radiation-induced chromosomal instability in BALB/c and C57BL/6 mice: the difference is as clear as black and white, Radiat. Res. 147 (1997) 121–125.
[107] L.C. Dugan, J.S. Bedford, Are chromosomal instabilities induced by exposure of cultured normal human cells to low- or high-LET radiation? Radiat. Res. 159 (2003) 301–311.
[108] K. Hamasaki, Y. Kusunoki, E. Nakashima, Clonally expanded T lymphocytes from atomic bomb survivors in vitro show no evidence of cytogenetic instability, Radiat. Res. 172 (2009) 234–243.
[109] W.F. Morgan, M.B. Sowa, Non-targeted bystander effects induced by ionizing radiation, Mutat. Res-Fund Mole Mech. 616 (2007) 159–164.
[110] W.F. Morgan, W.J. Bair, Issues in low dose radiation biology: the controversy continues. Perspective, Radiat. Res. 179 (2013) 501–510.
[111] S. Wolff, Adaptation of human and other mammalian cells to low doses of radiation, Radiat. Res. 141 (1995) 115–117.
[112] E.I. Azzam, S.M. de Toledo, G.P. Raaphorst, R.E.G. Mitchel, Low-dose ionizing radiation decreases the frequency of neoplastic transformation to a level below the spontaneous rate in C3H 10T1/2 cells, Radiat. Res. 146 (1996) 369–373.
[113] B.R. Scott, A biological-based model that links genomic instability, bystander effects, and adaptive response, Mutat. Res. 568 (2004) 129–143.
[114] A.M. Hooker, M. Bhat, T.K. Day, J.M. Lane, S.J. Swinburne, A.A. Morley, P.J. Sykes, The linear no-threshold model does not hold for low-dose ionizing radiation, Radiat. Res. 162 (4) (2004) 447–452.
[115] D.G. Hoel, J.K. Hasemann, M.D. Hogan, J. Huff, E.E. McConnell, The impact of toxicity on carcinogenicity studies: implications for risk assessment, Carcinogenesis 9 (1988) 2045–2052.
[116] National Council for Radiation Protection and Measurements (NCRP), Liver Cancer Risks from Internally-deposited Radionuclides vol 135, NCRP Report No, Bethesda, MD, 2001.
[117] S.A. Amundson, M. Bittner, A.J. Fornace, Functional genomics as a window on radiation stress signaling, Oncogene 22 (2003) 5828–5833.
[118] G.J. Kim, G.M. Fiskum, W.F. Morgan, A role for mitochondrial dysfunction in perpetuating radiation-induced genomic instability, Cancer Res. 66 (2006) 10377–10383.
[119] D.R. Spitz, E.I. Azzam, J.J. Li, D. Gius, Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: a unifying concept in stress response biology, Cancer Metastasis Rev. 23 (2004) 311–322.
[120] D. Gius, D.R. Spitz, Redox signaling in cancer biology, Antioxidants Redox Signal. 9 (7&8) (2006) 1249–1252.
[121] A.R. Kennedy, J. Guan, J.H. Ware, Countermeasures against space radiation induced oxidative stress in mice, Radiat. Environ. Biophys. 46 (2007) 201–203.
[122] E.I. Azzam, S.M. de Toledo, D.R. Spitz, J.B. Little, Oxidative metabolism modulates signal transduction and micronucleus formation in bystander cells from alpha-particle-irradiated normal human fibroblast cultures, Cancer Res. 62 (2002) 5436–5442.
[123] R. Lall, S. Ganapathy, M. Yang, S. Xiao, T. Xu, H. Su, M. Shadfan, J.M. Asara, I. Ben-Sahra, B.D. Manning, J.B. Little, Z.-M. Yuan, Low-dose radiation exposure induces a HIF-1-mediated adaptive and protective metabolic response, Cell Death Differ. 4 (2014) 1–9.
[124] A.E. Waltar, A.L. Brooks, J.M. Cuttler, L.E. Feinendegen, A.J. Gonzalez, W.M. Morgan, The high price of public fear of low-dose radiation, J. Radiol. Prot. 36 (2016) 387.
[127] R.D. Evans, A.J. Keane, B.J. Hasterlik, A.T. Keane, R.J. Kolenkow, W.R. Neal, M.M. Shanahan, The risk of bone cancer in man from internally deposited radium, Br. J. Radiol. 41 (485) (1968) 391–393.
[129] Strahlenschutzkommission (SSK), Dose and dose-rate effectiveness factor (DDREF) recommendationas by German commission on radiological protection with scientific grounds: chair: Beckow J. Members, in: M. Atkinson, G. Dietze, J. Kiefer, W. Ruhm (Eds.), Adopted at the 268th Meeting of the German Commission on Radiological Protection, 2014, pp. 13–14 February 2014.
[130] G.N. Taylor, R.D. Lloyd, C.W. Mays, Promotion of radiation-induced liver neoplasia by ethanol, Health Phys. 62 (1993) 178–182.
[131] B.E. Rogers, R.J. Baker, Frequencies of micronuclei in bank voles from zones of high radiation at Chernobyl, Ukraine, Environ. Tox. Chem. 6 (2000) 1644–1648.
[132] D.L. Portess, G. Bauer, M.A. Hill, P. O'Neill, Low-dose irradiation of non-transformed cells stimulates the selective removal of precancerous cells via intercellular induction of apoptosis, Cancer Res. 67 (2007) 1246–1253.
[133] J.L. Mauderly, J.A. Pickrell, C.H. Hobbs, The effects of inhaled 90 Y fused clay aerosol in pulmonary function and related parameters of the beagle dog, Radiat. Res. 56 (1973) 83–96.

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BEIR VI radon: The rest of the story

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ABSTRACT

The National Academy of Sciences (USA) conducted an extensive review on the health effects of radon (BEIR VI). This was a well written and researched report which had impact on regulations, laws and remediation of radon in homes. There were a number of problems with the interpretation of the report and three are focused on here. First, most of the radiation dose used to estimate risk was from homes with radon levels below the US Environmental Protection Agency's action level so that remediation had minor impact on total calculated attributable risk. Remediation of the high level homes (i.e., above the action level) would therefore have a minor impact on the calculated "population attributable risk". In individual homes with very high levels of radon, remediation may minimally reduce individual risk. Second, the conclusion communicated to the public, regulators and law makers was "Next to cigarette smoking radon is the second leading cause of lung cancer." This is not an accurate evaluation of the report. The correct conclusion would be: Next to cigarette smoking, high levels of radon combined with cigarette smoking is the second leading cause of lung cancer. In the never-smokers, few cancers could be attributable to radon. Thirdly, there is little question that high levels of radon exposure in mines combined with cigarette smoke and other significant insults in the mine environment produces excess lung cancer. However, the biological responses to low doses of radiation are different from those produced by high levels and low doses may result in unique protective responses (e.g. against smoking-related lung cancer). These three points will be discussed in detail. This paper shows that in contrary to the BEIR VI report, risk of lung cancer from residential radon is not increased and radon in homes appears to be helping to prevent smoking-related lung cancer. Thus, laws requiring remediation of homes for radon are providing little if any public health benefits.

1. Introduction

Radon is a naturally occurring colorless and odorless monatomic nobel gas that is part of the natural radioactive decay series starting with uranium-238 (^{238}U). Under standard conditions radon has very little chemical reactivity; therefore inhalation of radon alone has little biological effect. However, Radium-226 decays with a complex decay chain to produce radon-222 (^{222}Rn) which has a 3.82 day half-life and produces a range of short lived daughters that release alpha, beta and gamma radiation when they decay to become stable lead. The detailed decay chain is outlined in BEIR VI [1] as well as in numerous other publications. Even though radon is a noble gas, the radon daughters are charged and attach to small airborne particles in the environment so that they can be inhaled and deposited in the respiratory tract including the deep lung. The "attached fraction" and the "equilibrium" are both important variables which depend on the environment where the radon is released. These factors impact the local absorbed radiation doses throughout the respiratory tract [1]. To characterize exposure to Uranium miners and individuals in homes, a metric was developed which is called a Working Level Month (WLM). This is the product of the average (over time) radon concentration and duration of exposure in the radioactive environment. It is important to be able to convert WLM into a radiation absorbed dose in Gy to the lung, or equivalent dose in Sv to the lung, or effective whole-body dose in Sv (or related units). This approach has been used in BEIR VI for estimating radiation absorbed dose to the lung through the use of biological dosimetry [2–6] and dosimetric modeling. The central estimate obtained was 1.0 Gy/WLM [1]. However, uncertainty related to this value appears not to have been addressed, thereby limiting reliable uncertainty characterization for radiation absorbed dose estimates.

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There is little question that high radon concentrations in uranium mines and related absorbed radiation doses to the respiratory tract combined with the worker environment and worker lifestyle resulted in an increase in lung cancer [1,7–9]. The radiation dose estimates and lung cancer data for uranium miners were combined with the dose derived from within-home radon exposure to estimate lung cancer risk from radon inhalation for a range of exposure levels [1,10,11]. Epidemiology research has been combined with multivariate risk models to demonstrate the influence of radon in homes on lung cancer frequency. Use of multivariate models allows for addressing multiple risk factors and making adjustments for some covatiate influences. Additional research employing meta-analysis of data from multiple studies has led to the claim of evidence for an increase in lung cancer risk as radon levels in homes increase [11–17]. This information supports the basis for current risk estimates for radon in homes and is claimed to be consistent with the values of risk derived using the uranium miner data. Based on the result of the indicated studies, the International Commission on Radiation Protection (ICRP) has revised its lung cancer risk recommendations. They have suggested: "a lifetime excess absolute risk of 5×10^{-4} per WLM [14×10^{-5} per (mJh/m^3)] should now be used as the nominal cancer risk coefficient for radon-and radon-progeny induced lung cancer, replacing the previous *Publication 65* [18] value of 2.8×10^{-4} per WLM [8×10^{-5} per (mJh/m^3)]." The ICRP further suggested that radon be regulated based on dose to the respiratory track to make it consistent with other internally deposited radionuclides [19]. The power of epidemiological studies has been reviewed to determine what we really know about low dose radiation biology [20]. It was determined that the risk in the low dose range and the shape of the dose-response relationship in the dose range experienced in homes is difficult to define from epidemiology studies and requires large sample sizes [20]. Combining data from multiple studies is claimed by epidemiologists to help to reduce uncertainties but as demonstrated in a recent study [21], uncertainty appears to significantly "increase" when using combined datasets.

The claimed induction of lung cancer by low absorbed radiation doses from radon seems to be at odds with the animal studies where very large doses of alpha emitters to the lungs were required to increase the frequency of lung cancer [22–26]. Similar animal and human studies showed that the bone and liver required large doses from low dose rate internally deposited radionuclides to increase cancer frequency [26–28]. The deposition and non-uniform distribution of the internally incorporated radionuclides in the lungs, liver and bone may in part be responsible for the high doses required to induce cancer since most of the protective functions are not exposed to radiation as is the case for uniform whole body high dose rate exposure. Another major difference between the experimental animal studies and humans that may influence the cancer frequency are environmental and life style factors like cigarette smoking in humans. This seems to be the major factor responsible for these large differences. Radiation in the absence of smoking seems to be much less carcinogenic than is observed with the multiplicative [19] or super additive [1] interaction between cigarette smoking and radon exposure.

The potential for a marked interaction in a protective way (e.g., adaptive response) between cigarette smoking and radon in homes was suggested in epidemiological studies using an ecological approach. These ecological studies involving humans show that using USA county by county radon measurements and lung cancer data the higher the radon activity and dose in homes the lower the lung cancer incidence. Such data were the basis for postulating a hormetic or protective effect from low doses of radon [29–34]. Efforts have been made to explain the apparent protective inverse relationship between radon concentrations in homes and lung cancer data published by Cohen [29] displaying a hormetic exposure-response relationship. Similar low dose radiation protective effects were obtained in animal studies using injected cigarette smoke carcinogen (BPDE) to produce multiple lung tumors per animal and using low dose gamma rays (fractionated exposure) to repeatedly stimulate anticancer immunity [35].

The influence of smoking as a confounder for radon induced lung cancer was reviewed [36]. The researcher stated the following: "In general, quantitatively similar, strong negative correlations are found for cancers strongly linked to cigarette smoking, weaker negative correlations are found for cancers moderately increased by smoking while no such correlation is found for cancer not linked to smoking." These observations were taken as an explanation (without any scientific backing) to account for the negative correlation that Cohen observed between smoking and radon across counties. An alternative and science-based explanation would be that low doses of radiation activate molecular and cellular processes that act to protect against lung cancer and decrease the cancer frequency. These mechanisms are discussed in other papers in this Special Issue [37,38]. Extensive molecular, cellular and other data seem to support this hypothesis [37,39].

The mechanistic data on the interaction between radiation and molecular and cellular processes which are protective against cancer formation in the low dose and dose-rate region has been reviewed [40]; Tharmalingam under review). These findings will be briefly summarized in this manuscript. The question that remains is the effect of low doses of radon in homes. The use of modern molecular and cellular biology may be able to shed some light on this important question.

The risk values derived from the linear extrapolation of the miner data to the levels observed in homes was compared to the data derived from a number of studies of radon induced cancers in homes and were said to be consistent and adequately represent the data. It is important to remember that the radon exposure to the general population in homes is a thousand-fold to a hundred-fold less than those in most mines. This makes this extrapolation questionable and would require that the biological mechanisms of action for production of lung cancer following high doses of radon are the same as those for low doses. It has however now been established that high radiation doses suppress natural defenses (barriers) against cancer facilitating cancer occurrence while low doses enhance these cancer barriers [37]; in this Special Issue).

2. Results

We have focused on three major problems associated with the interpretation of the BEIR VI report. These are briefly discussed.

2.1. Problem 1

The first Problem was the summing of individual-specific doses to the target organ of interest and this included many individuals from homes below the radon action level. The resultant summed individual-specific doses is called collective dose and use of collective dose implies LNT is being used as the null hypothesis. The use of LNT as a null hypothesis irrespective of the data essentially guarantees an LNT outcome since the inclusion of high-dose data guarantees a positive slope with a locked intercept [37]. Here, the intercept location does not reflect the variability in the low dose range. This setup makes it difficult to reject the null hypothesis [41,42]. Therefore, collective dose cannot be reliably applied to threshold or other nonlinear dose-response relationships. In BEIR VI the distribution of radon concentrations in homes was characterized based on radon measurements and was converted to corresponding radiation absorbed doses (i.e., unweighted dose) using a dose conversion (from WLM to absorbed dose) factor [43]. The sum (collective dose) of all the individual-specific absorbed radiation doses to the respiratory tract in homes was used as the population-level dose metric. These data are summarized in Tables 3–8 on page 95 of BEIR VI [1]. It was observed that approximately 95% of all homes had radon levels below the EPA action limit of 4 pCi/l or 148 Bq/m^3. Homes, below the EPA action level for radon remediation, were the major contributor of calculated collective dose for the respiratory tract. Thus, radon remediation would have minimal impact on the collective dose

Table 1
Tables 3–8 pg.95 of the BEIR VI report: Distribution of attributable risks for U.S. males from indoor residential radon exposure, based on BEIR VI models.

Exposure range, BqM^{-3}	Proportion of homes in Range, %	Contribution to AR			
		Exposure-age-contribution model		Exposure-age-duration model	
		Actual	%	Actual	%
0–25	49.9	0.018	12.8	0.013	12.8
26–50	23.4	0.026	18.5	0.018	18.4
51–75	10.4	0.02	14.2	0.014	14.2
76–100	5.4	0.015	10.5	0.01	10.5
101–150	5.2	0.02	13.9	0.014	13.9
151–200	2.4	0.013	9.2	0.009	9.2
201–300	1.8	0.014	9.6	0.01	9.7
301–400	0.7	0.007	5.2	0.005	5.3
401–600	0.4	0.006	4.5	0.005	4.6
601 +	0.4	0.002	1.5	0.002	1.6
Total	100	0.141	100	0.099	100

(sum of cumulative absorbed doses to individuals) or the "calculated" LNT-based radon-associated cancer risk.

In the BEIR VI [1] report the LNT model was forcibly employed using collective dose based on the measured distribution of radon in homes as already indicated. The collective dose was used as the independent variable for population indoor residential radon exposure which as multiplied by a lung cancer risk coefficient based on uranium mines exposed to high-level radon. This approach was claimed to be valid and the models and the assumptions used in the report were based on the claimed best scientific data available at the time. In fact the risk assessment approach used in BEIR VI essentially guarantees and LNT outcome irrespective of the nature of the low-dose data. Furthermore, the Health Physics Society recommends that collective dose is not used for populations in which almost all individuals are estimated to receive an individual dose under 50 mSv in one year or a lifetime dose of less than 100 mSv above background [44].

A major Problem in the BEIR VI report for using collective dose is illustrated in Table 1 (Tables 3–8 Page 95). This table uses the collective dose in homes combined with BEIR VI models to calculate attributable risk of lung cancer to US males from indoor residential radon. It was noted that the risk distributions were similar for females. Fig. 1 illustrates that only 5.7% of the homes had radon levels above the EPA action level and that about 30% of the calculated attributable risk came from these homes. Thus, 70% of the calculated risk, more than 90% of which came from smokers, would be influenced by remediation of each and every home with radon above the EPA action level.

Collective dose information was therefore combined with the LNT-based lung cancer risk coefficient derived using high-dose uranium miner data and employed for low-dose, in-home radon exposure [1]. This was done for smokers and non-smokers, women and men using two different multivariate models (exposure-age-concentration and exposure-age duration). These models formed the basis for the risk estimates reported in BEIR VI. The justification for the use of the LNT in this report is based on the information available at the time so not based on current knowledge. The dose to a cell from a single traversal of an alpha particle can be calculated to be about 0.20 Gy and in the low dose region where few cells have multiple traversals would be constant regardless of the absorbed dose to the organ. Thus, in this low dose region as the absorbed dose decreases the main change would be the number of cells directly "hit" and this number would decrease as a linear function of absorbed radiation dose. This report was written before a great deal of research was conducted in the low dose range and before the development of the microbeam that made it possible to evaluate the response of "hit" cells and "bystander cells".

2.2. Problem 2

The second Problem with the BEIR VI report was the main conclusion that was widely accepted and used. The message was, next to cigarette smoking radon is the second leading cause of lung cancer. The data from BEIR VI page 97 (Tables 3–10) provides an excellent summary of the "Estimated number of lung cancer deaths in the U.S. in 1995 attributable to indoor residential radon progeny exposure". This table shows that lung cancer is a frequent disease with a total of 157,400 deaths in males and females combined during this year. In males, about 95% of lung cancers occurred in males that smoke cigarettes and about 90% of the females with lung cancer were smokers. In the never smoker category the range of cancers attributable to radon was 1200–2900. Cancers attributable to radon plus cigarette smoking using the different models for calculation ranged from 15,400–22,300. There is no reason to favor either end of this range of lung cancer estimates. To express these numbers in a graphic form the mean values were used [45] and is shown in Fig. 1. This figure makes it very clear that radon alone is calculated to produce only 1.3% of the total lung cancers and does not account for a large number of excess lung cancer. It strongly illustrates that the statement commonly used to describe radon induced lung cancer, "next to cigarette smoking, radon is the second leading cause of lung cancer" is not accurate. High levels of radon combined with cigarette smoking results in an increase of 12% of the total lung cancers and thus, may be the second leading cause of lung cancer. This graph also illustrates that if everyone with radon in their homes would stop smoking there would have been 135,300 fewer lung cancers predicted in this year. If everyone with radon in their homes had all the radon remediated, which is not possible because radon is present both indoors and outdoors, there would be an LNT-based calculated total of about 18,000 less cancers with only 1970 of those calculated to have been produced in non-smokers [31]. The conclusion that can be drawn from this discussion is that if you have radon in your home stop smoking and do not remove radon because if you continue to smoke the radon may help prevent lung cancer occurrence [31]. If a person smokes and has a high level of radon in their home, active and expensive mitigation would be recommended and could result in a significant decrease in their lung cancer risk attributable to smoking combined with radon. If a person has radon in their home and they are a non-smoker they would have very little increased risk for lung cancer. If a person has a very high level of radon in their home and they are a non-smoker then perhaps they could institute a radon mitigation program, even as simple as opening windows and putting a fan in their basement. This discussion along with the discussion of problem 1 illustrates that remediation of radon in homes and in public buildings like schools where there is no smoking allowed would be expected to have little impact on public health and perhaps the laws requiring it before selling a home should be re-evaluated.

Inhalation of other environmental materials that cause a chronic lung disease may increase lung cancer risk. It has been shown that inhalation of diesel exhaust at high concentrations results in an overloading of the lungs with particulates and results in a chronic lung disease that increases the risk for lung cancer. Early in the research this was thought to be related to chemical carcinogens associated with the diesel particles. However, additional research demonstrated that inhalation of high levels of carbon black particulate, without any chemical carcinogens, resulted in the same response [46]. This suggests that any high level insult to the lung which creates the potential for a chronic inflammatory disease results in an environment that increases lung cancer risk and may be responsible for the synergistic interaction seen between cigarette smoke and radon exposure [28]. Interestingly low-dose radiation such as from inhaled radon suppresses disease promoting inflammation [47].

Early in the uranium mining history worldwide (including Canada), many of the miners were required to inhale aluminum oxide (McIntyre Powder) daily before entering the mines as a "protection" against

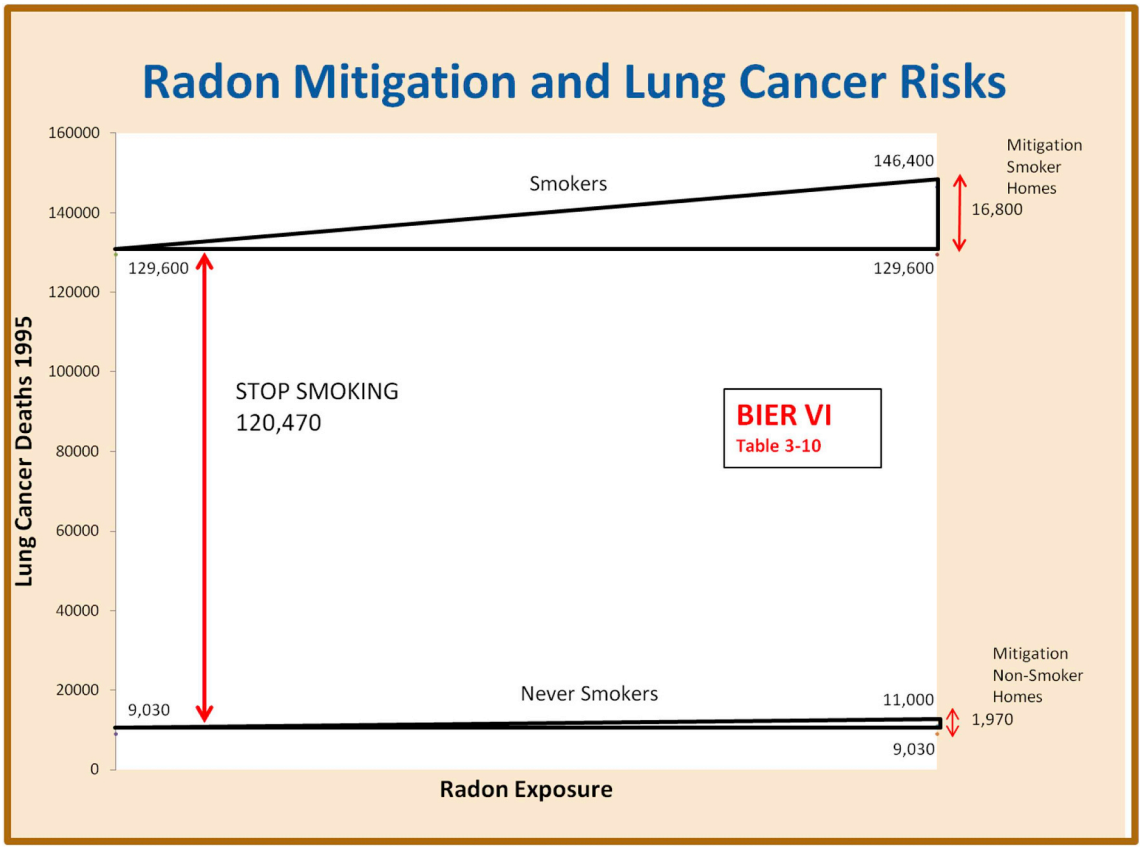

Fig. 1. Radon mitigation and lung cancer risks. Radon alone is calculated to produce only 1.3% of the total lung cancers and does not account for a large number of excess lung cancer. High levels of radon combined with cigarette smoking results in an increase of 12% of the total lung cancers and thus, may be the second leading cause of lung cancer.

silicosis in the lung [48]. It has been demonstrated that silica generates free radicals which may provide a mechanism for the induction of both chronic lung disease and cancer. There has been a significant amount of research focused on the molecular mechanisms of silica induced free radical production and its contribution to carcinogenesis [49–53]. Silica can spontaneously produce ROS in aqueous solution, as well as stimulate phagocytic cells to generate ROS [54]. Silica has also been shown to generate lipid peroxidation products that can damage DNA [52]. The hydroxyl (OH) radical can be very effective at damaging DNA, although very short lived. The OH radical is by far the most potent ROS to react with DNA, generating multiple products from all four bases. OH radicals can be produced in several ways; in biological systems the Fenton reaction is the most significant. Fenton reactions are generally defined by the involvement of transition metals and hydrogen peroxide. Often iron impurities are present in aqueous solution or on the surface of the silica crystals, and through Fenton reactions, can greatly contribute to OH radical production. Silica in an aqueous environment will naturally produce O_2 radicals, and through Haber-Weiss reactions, generate OH radicals as well [55]. Because free silica can readily bind to the phosphate backbone of DNA, the site of OH radical generation will be in close proximity the DNA. The localized silica greatly increases the effectiveness of the short lived OH radical to damage DNA via Fenton chemistry [50].

Concern about the pro-oxidant activity of aluminum (McIntyre Powder) has raised a number of questions about the potential of this material to induce changes in the reactive oxygen status of the lung, induce chronic inflammatory disease and interact with radon and modify the risk for lung cancer [28,56]. Exley [56] hypothesized that an aluminum radical may exist based on overwhelming evidence supporting its pro-oxidant properties. The evidence suggests that aluminum can exist as a free radical in aqueous solution and has pro-oxidant properties. The ability of aluminum to catalyze iron driven biological oxidation has been reviewed [56]. Iron-driven oxidation of Fe^{2+} is a strong biological oxidizer because it reduces O_2 to an O_2 radical. Therefore, the ability of a molecule to reduce Fe^{3+} and maintain excess Fe^{2+} should promote iron driven oxidation. Exely [56] suggests that the potential aluminum radical facilitates the reduction of Fe^{3+} to Fe^{2+} which maintains the concentration of Fe^{2+} and supports oxidation by hydrogen peroxide. The characteristics of aluminum that enable maintenance of excess Fe^{2+} will greatly support Fenton reactions and can produce vast amounts of the genotoxic OH radical. In many cases Ontario uranium miners were exposed to a combination of high doses of radon gas, large quantities of both freshly fractured silica dust and aluminum powder (as a prophylactic treatment for silicosis). Collectively, lung exposure to these agents may have caused an imbalance between pro-oxidants and anti-oxidants, where pro-oxidants were favored. This pro-oxidant imbalance has the potential to impair the body's ability to protect against oxidative injury. Although additional research in this important area is required, it is suggested that there exists a synergistic relationship between radon gas, silica, and aluminum in the lung which greatly increases lung cancer risk when exposed to all three compared to radon alone.

2.3. Problem 3

The third Problem addressed here is the apparent disconnect in the predicted cancer risks in the low dose region derived from human epidemiologic studies, and lung cancer risks derived from experimental animal studies and is suggested by mechanistic studies. This is especially true for risks from internally deposited radioactive material in experimental animals and those predicted from epidemiologic data from studies of human populations. The human data are often based on

study designs that favor an LNT outcome [37,57] and thereby suggest elevated risks in the low dose region where as the extensive laboratory research on the effects of internally deposited radioactive material support cancer induction only with very high doses. The experimental animal data was carefully reviewed in the late 1980's and showed that large concentrations of internally deposited radioactive materials, which resulted in large absorbed radiation doses, were required to produce cancer [22,58,59]. These data have been re-evaluated and similar conclusions reached for many cancer types produced by either high or low LET radiation following internal deposition of radioactive materials including radon [23–27,45,60]. The risks from radon represent a prime example of these differences. For radon it seems that a major cause of the differences between the human data and experimental animal data is related to the interaction between high-level radon and smoking and life style in humans. This interaction seems to be greater than additive [1] for high-level radon and may even multiply the risk [19]. This may relate to high-level radon being immunosuppressive unlike for low-level radon which seems to stimulate anticancer immunity and to protect from smoking-related lung cancer, as evidenced by hormetic dose-response relationships [31]. Without smoking, as is the case in the experimental animal studies, the risk from high-level radon is greatly reduced and there may be no risk increase for low-level radon.

It has also been well documented that the response at the molecular and cellular level change as a function of both dose and dose-rate and require major paradigm shifts in the field of radiation biology [40,45,61]; Tharmalingham under review). Following high doses and dose rates the molecular responses seem to be involved in pathways that are detrimental. The responses after a low dose and dose rate exposure suggest activation pathways that result in protective processes. Such differences point out the need for a paradigm change away from the linear no threshold model to models that reflect the potential for protective adaptation and hormesis. The hormetic response should be considered in risk assessment and modeling which makes it necessary to have negative terms and/or thresholds in these models [31,32,62,63]. These differences must be understood to provide adequate and appropriate protection from internally deposited radioactive materials [64].

The amount of research conducted at the molecular and cellular level for high LET alpha particles such as found following radon exposures is rather limited. The data from high LET radiation exposures has been associated with the use of microbeams and the discovery of the “bystander” effects or the influence of cell/cell and cell/tissue communication. With the development of the microbeam it became possible to know the individual cells that were “hit” and had energy deposited in them. This made it possible to observe first-hand the fact that the cells with energy deposited in them were not the only cells that responded to the radiation exposure. Such observations suggested the need for a major paradigm shift in radiation biology away from the “hit theory” to more of a tissue response to high LET radiation [6,65,66]. It became obvious that the whole tissue is responding to radiation from high LET radiation [67]. In addition to the whole tissue responding in a protective manner it became obvious that exposure to radon initiated responses in the whole organism. Radon deposits almost all its energy in the respiratory tract so it was thought that this exposure would produce little response outside the exposed organs. Studies on the response of the immune system to radon exposure demonstrated that cells and tissues from the immune system, outside the directly exposed tissue, were also responding to the radon exposure even though there was little radiation dose to these tissues [68].

Two different approaches have been suggested to incorporate mechanistic studies into regulatory processes. First is the use of a systems biology approach [69] which would be based on an integration of all the data from the molecular to the whole-body level. This is the ultimate goal for standard setting but still requires a great deal of research and communication. The second approach is to determine the responses (both protective and deleterious) as a function of dose and dose rate in the key events in the critical pathways for the induction of cancer [70]. With further research to support these approaches both will provide input into standards setting and result in standards based on the best scientific data and best models available.

At the molecular level it has been carefully documented that DNA damage increases as a linear function of radiation dose. The repair and removal of DNA damage is non-linearly related to dose and has been carefully reviewed [38] in this Special Issue). This demonstrates that low doses and low dose rates do not result in increased levels of DNA damage or induction of mutations. Low dose and dose-rate exposure also activates a number of molecular pathways that change gene, protein and miRNA expression. The pathways involved in many of these changes in the low dose region seem to be protective. At the cellular level low doses result in cell cycle arrest, senescence and selective apoptosis which remove damaged cells from the population suggesting a decrease in cancer risk. Finally, at the whole organ and tissue level bystander effects, tumor suppression, modification of antioxidation states and mediation of the immune system are also thought to be protective. All these responses together at every level of biological organization provide strong support that would suggest that radon exposure at low levels would not elevate cancer frequency. However, if high-level radiation is present in the presence of other insults, like cigarette smoking the frequency of cancer has been shown to increase, while low-level radiation has been shown to reduce lung cancer risk.

3. Summary

This manuscript demonstrates, as illustrated in the BEIR VI report, that remediation of radon in homes has minimal impact on total collective dose, and that most of the dose in the calculation comes from homes where the radon level is below the EPA action level. Therefore, the risk assessments remediation would be predicted to have minimal impact on public health. Thus, the laws that require radon testing in homes prior to selling should be focused on homes with much higher levels of radon, where there is little question as to the potential increase in lung cancer in smokers.

The conclusion from BEIR VI that radon exposure is the second leading cause of lung cancer is not accurate. The real conclusion from BEIR VI is first, that remediation has little impact on radon risk in homes. Second, that extensive data shows that high-level radon interacts with other environmental insults in a more than additive way to increase cancer risk while low-level radon seems to protect from inflammatory diseases. These environmental insults to the lung such as smoking, diesel exhaust, mine dust and perhaps inhalation of any particulate material like aluminum that are pro-oxidants [56] all seem to result in a chronic inflammatory disease [28]. The inflammatory disease interacts with the radiation from high-level radon in more than an additive way to increase lung cancer risk. The more accurate statement from the BEIR VI report would have to be that high-level radon combined with cigarette smoking may be the second leading cause of lung cancer. Since more than ninety percent of lung cancers are present in smokers, the increased risk for lung cancer detected in epidemiology studies in homes may be related to the interaction between high-level radon and smoking and not to radon alone.

Studies demonstrated that cancers that are closely linked to smoking decrease in frequency as a function of increasing radon dose [36] in a manner similar to that seen for lung cancer [29]. This observation was taken to suggest cigarette smoking was a confounder that resulted in the negative slope observed in the ecological studies conducted by Cohen [29]. Another way to interpret these results would be to suggest that radon exposures alter gene expression and metabolic pathways that result in adaptive protective changes. Such a hypothesis is supported by mechanistic studies which suggest that low doses of radiation induce unique adaptive protective molecular and cellular changes. The biological responses induced by low dose and dose rate exposures are very different from those following high doses. Many of these responses may

be protective and suggest that in the low dose region the radiation risk does not increase linearly with dose. This very large data base outlined in this Special issue [38] makes it essential to consider the existence of thresholds (for elevated cancer risk) and perhaps even protective/beneficial responses in the low dose range. These data suggest the need for another paradigm change away from the linear no threshold dose response models toward either threshold or protective/beneficial response models.

Radiation standards and radiation risk assessment should be based on the best scientific data and models available and reflect modern radiobiology. However, reliance on findings of unreliable epidemiologic studies rather than finding from modern basic and applied research unfortunately seems to be the preference of organizations such as the National Council on Radiation Protection and Measurements, Environmental Protection Agency, International Commission on Radiological Protection, and United Nations Scientific Committee on the Effects of Atomic Radiation.

Declarations of interest

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References

[1] NRC/NAS, Report No. 135, Liver Cancer Risk from Internally-deposited Radionuclides, National Council on Radiation Protection and Measurements, Bethesda Md, 1999 20814.
[2] A.L. Brooks, K. Rithidech, N.F. Johnson, D.G. Thomassen, G.J. Newton, Evaluating chromosome damage to estimate dose to tracheal epithelial cells, Twenty-ninth Hanford Life Sciences Symposium. Indoor Radon and Lung Cancer: Reality or Myth? Hanford Press, RichlandWa, 1990, pp. 601–614 Part 2.
[3] A.L. Brooks, S. Bao, P.W. Harwood, B.H. Wood, W.B. Chrisler, M.A. Khan, R.A. Gies, F.T. Cross, Induction of micronuclei in respiratory tract following radon inhalation, Int. J. Radiat. Biol. 72 (5) (1997) 485–495.
[4] A.L. Brooks, M.A. Khan, A. Duncan, R.L. Buschbom, R.F. Jostes, F.T. Cross, Effectiveness of radon relative to acute 60Co gamma-rays for induction of micronuclei in vitro and in vivo, Int. J. Radiat. Biol. 66 (6) (1994) 801–808.
[5] M.A. Khan, F.T. Cross, R. Jostes, E. Hui, J.E. Morris, A.L. Brooks, Micronuclei induced by radon and its progeny in deep-lung fibroblasts of rats in vivo and in vitro, Radiat. Res. 139 (1) (1994) 53–59.
[6] J.M. Nelson, A.L. Brooks, N.F. Metting, M.A. Khan, R.L. Buschbom, A. Duncan, R. Miick, L.A. Braby, Clastogenic effects of defined numbers of 3.2 MeV alpha particles on individual CHO-K1 cells, Radiat. Res. 145 (5) (1996) 568–574.
[7] J.H. Lubin, Y.L. Qiao, P.R. Taylor, S.X. Yao, A. Schatzkin, B.L. Mao, J.Y. Rao, X.Z. Xuan, J.Y. Li, Quantitative evaluation of the radon and lung cancer association in a case control study of Chinese tin miners, Cancer Res. 50 (1) (1990) 174–180.
[8] J.M. Samet, Radon and lung cancer, J. Natl. Cancer Inst. 81 (10) (1989) 745–757.
[9] J.M. Samet, D.R. Pathak, M.V. Morgan, C.R. Key, A.A. Valdivia, J.H. Lubin, Lung cancer mortality and exposure to radon progeny in a cohort of New Mexico underground uranium miners, Health Phys. 61 (6) (1991) 745–752.
[10] J.H. Lubin, J.D. Boice Jr., Estimating Rn-induced lung cancer in the United States, Health Phys. 57 (3) (1989) 417–427.
[11] J.H. Lubin, L. Tomasek, C. Edling, R.W. Hornung, G. Howe, E. Kunz, R.A. Kusiak, H.I. Morrison, E.P. Radford, J.M. Samet, et al., Estimating lung cancer mortality from residential radon using data for low exposures of miners, Radiat. Res. 147 (2) (1997) 126–134.
[12] J. Chen, Estimated risks of radon-induced lung cancer for different exposure profiles based on the new EPA model, Health Phys. 88 (4) (2005) 323–333.
[13] J. Chen, Lifetime lung cancer risks associated with indoor radon exposure based on various radon risk models for Canadian population, Radiat. Protect. Dosim. 173 (1–3) (2017) 252–258.
[14] S. Darby, D. Hill, A. Auvinen, J.M. Barros-Dios, H. Baysson, F. Bochicchio, H. Deo, R. Falk, F. Forastiere, M. Hakama, et al., Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies, Br. Med. J. 330 (7485) (2005) 223.
[15] D. Krewski, J.H. Lubin, J.M. Zielinski, M. Alavanja, V.S. Catalan, R.W. Field, J.B. Klotz, E.G. Letourneau, C.F. Lynch, J.L. Lyon, et al., A combined analysis of North American case-control studies of residential radon and lung cancer, J. Toxicol. Environ. Health Part A 69 (7) (2006) 533–597.
[16] J.H. Lubin, Z.Y. Wang, J.D. Boice Jr., Z.Y. Xu, W.J. Blot, L. De Wang, R.A. Kleinerman, Risk of lung cancer and residential radon in China: pooled results of two studies, Int. J. Canc. 109 (1) (2004) 132–137.
[17] J.M. Samet, Radiation and cancer risk: a continuing challenge for epidemiologists, Environ. Health : a global access science source 10 (Suppl 1) (2011) S4.
[18] ICRP, Memorandum from the British committee on radiation units and measurements. Advice following ICRP publication 60, Br. J. Radiol. 66 (792) (1993) 1201–1203.
[19] ICRP, Lung Cancer Risk from Radon and Progeny and Statement on Radon, International Commission of Radiological Protection, 2010.
[20] D.J. Brenner, R. Doll, D.T. Goodhead, E.J. Hall, C.E. Land, J.B. Little, J.H. Lubin, D.L. Preston, R.J. Preston, J.S. Puskin, et al., Cancer risks attributable to low doses of ionizing radiation: assessing what we really know, Proc. Natl. Acad. Sci. U.S.A. 100 (24) (2003) 13761–13766.
[21] L. Dobrzyński, K.W. Fornalski, J. Reszczyńska, Meta-analysis of thirty-two case–control and two ecological radon studies of lung cancer, J. Radiat. Res. 59 (2) (2017) 15.
[22] W.J. Bair, D.H. Willard, Plutonium inhalation studies. IV. Mortality in dogs after inhalation of Pu239O2, Radiat. Res. 16 (1962) 811–821.
[23] A.L. Brooks, P.E. Eberlein, L.A. Couch, B.B. Boecker, The role of dose-rate on risk from internally-deposited radionuclides and the potential need to separate dose-rate effectiveness factor (DREF) from the dose and dose-rate effectiveness factor (DDREF), Health Phys. 97 (5) (2009) 458–469.
[24] B.A. Muggenburg, R.A. Guilmette, F.F. Hahn, J.H. Diel, J.L. Mauderly, S.K. Seilkop, B.B. Boecker, Radiotoxicity of inhaled (239)PuO(2) in dogs, Radiat. Res. 170 (6) (2008) 736–757.
[25] J.F. Park, C.R. Watson, R.L. Buschbom, G.E. Dagle, D.J. Strom, R.E. Weller, Biological effects of inhaled 239PuO2 in Beagles, Radiat. Res. 178 (5) (2012) 447–467.
[26] O.G. Raabe, Concerning the health effects of internally deposited radionuclides, Health Phys. 98 (3) (2010) 515–536.
[27] NCRP, Liver Cancer Risk from Internally-deposited Radionuclides. Bethesda, Md, (2001), p. 135.
[28] S. Puukila, C. Thome, A.L. Brooks, G. Woloschak, D.R. Boreham, The role of radiation induced injury on lung cancer, Cancers 9 (7) (2017).
[29] B.L. Cohen, Test of the linear-no threshold theory of radiation carcinogenesis for inhaled radon decay products, Health Phys. 68 (2) (1995) 157–174.
[30] B.L. Cohen, G.A. Colditz, Tests of the linear-no threshold theory for lung cancer induced by exposure to radon, Environ. Res. 64 (1) (1994) 65–89.
[31] B.R. Scott, Residential radon appears to prevent lung cancer, Dose-response: a publication of International Hormesis Society 9 (4) (2011) 444–464.
[32] B.R. Scott, S.A. Belinsky, S. Leng, Y. Lin, J.A. Wilder, L.A. Damiani, Radiation-stimulated epigenetic reprogramming of adaptive-response genes in the lung: an evolutionary gift for mounting adaptive protection against lung cancer, Dose Response 7 (2) (2009) 104–131.
[33] J.S. Thompson, R. Weseman, F. Rochling, W. Grant, J. Botha, A. Langnas, D. Mercer, Radiation therapy increases the risk of hepatobiliary complications in short bowel syndrome, Nutr. Clin. Pract.: Off. Publ. Am. Soc. Parent. and Enteral Nutrit. 26 (4) (2011) 474–478.
[34] R.E. Thompson, D.F. Nelson, J.H. Popkin, Z. Popkin, Case-control study of lung cancer risk from residential radon exposure in Worcester county, Massachusetts, Health Phys. 94 (3) (2008) 228–241.
[35] V.R. Bruce, S.A. Belinsky, K. Gott, Y. Liu, T. March, B. Scott, J. Wilder, Low-dose gamma-radiation inhibits benzo[a]pyrene-induced lung adenoma development in a/j mice, Dose-response : a publication of International Hormesis Society 10 (4) (2012) 516–526.
[36] J.S. Puskin, Smoking as a confounder in ecologic correlations of cancer mortality rates with average county radon levels, Health Phys. 84 (4) (2003) 526–532.
[37] B.R. Scott, A critique of recent epidemiologic studies of cancer mortality among nuclear workers, Dose-response : a publication of International Hormesis Society 16 (2) (2018) 9.
[38] S. Tharmalingam, S. Sreetharan, A.L. Brooks, D.R. Boreham, Re-evaluation of the Linear No-threshold (LNT) Model Using New Paradigms and Modern Molecular Studies, CBI, 2018 current special edition.
[39] NCRP, Commentary 24, (2015) Bethesda, Maryland, USA.

[40] L.T. Dauer, A.L. Brooks, D.G. Hoel, W.F. Morgan, D. Stram, P. Tran, Review and evaluation of updated research on the health effects associated with low-dose ionising radiation, Radiat. Protect. Dosim. 140 (2) (2010) 103–136.
[41] J.J. Cardarelli 2nd, B.A. Ulsh, It is time to move beyond the linear No-threshold theory for low-dose radiation protection, Dose-response : a publication of International Hormesis Society 16 (3) (2018) 1559325818779651.
[42] B.A. Ulsh, A critical evaluation of the NCRP COMMENTARY 27 endorsement of the linear no-threshold model of radiation effects, Environ. Res. 167 (2018) 472–487.
[43] N.H. Harley, Effect of residential radon decay product dose factor variability on reporting of dose, Health Phys. 114 (4) (2018) 398–407.
[44] Health Physics Statement, (2010).
[45] A.L. Brooks, Thirty-sixth Lauriston S. Taylor Lecture on radiation protection and measurements–from the field to the laboratory and back: the what ifs, wows, and who cares of radiation biology, Health Phys. 105 (5) (2013) 407–421.
[46] J.M. Mauderly, Lung Overload: the Dilemma and Opportunities for Resolution, (1996).
[47] B.R. Scott, Radiation-hormesis phenotypes, the related mechanisms and implications for disease prevention and therapy, Journal of cell communication and signaling 8 (4) (2014) 341–352.
[48] S.L. Rifat, M.R. Eastwood, D.R. McLachlan, P.N. Corey, Effect of exposure of miners to aluminium powder, Lancet 336 (8724) (1990) 1162–1165.
[49] A.M. Knaapen, P.J. Borm, C. Albrecht, R.P. Schins, Inhaled particles and lung cancer. Part A: Mechanisms, Int. J. Canc. 109 (6) (2004) 799–809.
[50] X. Shi, V. Castranova, B. Halliwell, V. Vallyathan, Reactive oxygen species and silica-induced carcinogenesis, J. Toxicol. Environ. Health B Crit. Rev. 1 (3) (1998) 181–197.
[51] X. Shi, M. Ding, F. Chen, L. Wang, Y. Rojanasakul, V. Vallyathan, V. Castranova, Reactive oxygen species and molecular mechanism of silica-induced lung injury, J. Environ. Pathol. Toxicol. Oncol. : official organ of the International Society for Environmental Toxicology and Cancer 20 (Suppl 1) (2001) 85–93.
[52] X. Shi, Y. Mao, L.N. Daniel, U. Saffiotti, N.S. Dalal, V. Vallyathan, Silica radical-induced DNA damage and lipid peroxidation, Environ. Health Perspect. 102 (Suppl 10) (1994) 149–154.
[53] A. Shukla, C.R. Timblin, A.K. Hubbard, J. Bravman, B.T. Mossman, Silica-induced activation of c-Jun-NH2-terminal amino kinases, protracted expression of the activator protein-1 proto-oncogene, fra-1, and S-phase alterations are mediated via oxidative stress, Cancer Res. 61 (5) (2001) 1791–1795.
[54] V. Vallyathan, J.F. Mega, X. Shi, N.S. Dalal, Enhanced generation of free radicals from phagocytes induced by mineral dusts, Am. J. Respir. Cell Mol. Biol. 6 (4) (1992) 404–413.
[55] B. Halliwell, J.M. Gutteridge, Role of iron in oxygen radical reactions, Methods Enzymol. 105 (1984) 47–56.
[56] C. Exley, The pro-oxidant activity of aluminum, Free Radical Biol. Med. 36 (3) (2004) 380–387.
[57] B. Sacks, G. Meyerson, J.A. Siegel, Epidemiology without biology: false paradigms, unfounded assumptions, and specious statistics in radiation science (with commentaries by inge schmitz-feuerhake and christopher busby and a reply by the authors), Biological theory 11 (2016) 69–101.
[58] J.N. Stannard, Radioactivity and Health: a History, Pacific Northwest National Laboratory, Springfield, VA, 1988.
[59] R.C. Thompson, Life-span Effects of Ionizing Radiation in the Beagle Dog, DOE Office of Health and Environmental Research DE-AC06-k76RLO1830, 1989 PNL-6822, UC-408.
[60] F.T. Cross, R.G.D.G. Palmer, R.H. Busch, R.L. Buschbom, Influence of radon daughter exposure rate, unattachment fraction, and disequlibrium on occurrence of lung tumors, Radiat. Protect. Dosim. 7 (1984) 381–384.
[61] A.L. Brooks, Paradigm shifts in radiation biology: their impact on intervention for radiation-induced disease, Radiat. Res. 164 (4 Pt 2) (2005) 454–461.
[62] E.J. Calabrese, Hormesis: from marginalization to mainstream: a case for hormesis as the default dose-response model in risk assessment, Toxicol. Appl. Pharmacol. 197 (2) (2004) 125–136.
[63] E.J. Calabrese, L.A. Baldwin, Hormesis: the dose-response revolution, Annu. Rev. Pharmacol. Toxicol. 43 (2003) 175–197.
[64] R.A.M. Yadav, A. Kumar, B.N. Pandey, Mechanism of carcinogenesis after exposure to actinide radionuclides: emerging concepts and missing links, J. Rad. Can. Res. 8 (2017) 20–34.
[65] M.H. Barcellos-Hoff, A.L. Brooks, Extracellular signaling through the microenvironment: a hypothesis relating carcinogenesis, bystander effects, and genomic instability, Radiat. Res. 156 (5 Pt 2) (2001) 618–627.
[66] C.R. Geard, G. Jenkins-Baker, S.A. Marino, B. Ponnaiya, Novel approaches with track segment alpha particles and cell co-cultures in studies of bystander effects, Radiat. Protect. Dosim. 99 (1–4) (2002) 233–236.
[67] A.L. Brooks, Evidence for 'bystander effects' in vivo, Hum. Exp. Toxicol. 23 (2) (2004) 67–70.
[68] M. Nagarkatti, P.S. Nagarkatti, A. Brooks, Effect of radon on the immune system: alterations in the cellularity and functions of T cells in lymphoid organs of mouse, J. Toxicol. Environ. Health 47 (6) (1996) 535–552.
[69] M.H. Barcellos-Hoff, S.V. Costes, A systems biology approach to multicellular and multi-generational radiation responses, Mutat. Res. 597 (1–2) (2006) 32–38.
[70] A.L. Brooks, D.G. Hoel, R.J. Preston, The role of dose rate in radiation cancer risk: evaluating the effect of dose rate at the molecular, cellular and tissue levels using key events in critical pathways following exposure to low LET radiation, Int. J. Radiat. Biol. 92 (8) (2016) 405–426.

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Mini-review

Review of the evidence for thresholds for DNA-Reactive and epigenetic experimental chemical carcinogens

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ABSTRACT

In the deliberations over many years on the question of thresholds for the carcinogenicity of chemicals, the dominant paradigm has been the linear no-threshold (LNT) model, derived from concepts formulated in radiation mutagenicity. Based on the analogy with radiation, the key mechanistic assumption underlying the assessment of the dose-effect of chemical-induced carcinogenicity has been that any dose, no matter how low, can lead to induction of mutations, which will result in some risk of neoplasia. The LNT assumption, however, was never well founded and, its application to chemical carcinogens, does not allow for differences in their disposition or mechanisms of action. These mechanisms include DNA-reactivity and epigenetic effects, resulting from very different properties of carcinogens, leading to different dose effects. This review of the research on dose effects of chemical carcinogens administered by repeat dosing for long duration reveals that only some experiments involving what are now recognized as DNA-reactive carcinogens yielded dose effects for induction of tumors which were consistent with the absence of a threshold (for 6/14 chemicals). None of these studies, however, included low doses documented not to produce genetic or other cellular toxicities that underlie carcinogenicity. Otherwise, most dose-effect experiments, including all with epigenetic agents (7), revealed no-observed-effect-levels for tumors, indicative of subthreshold doses. Based on highly informative experimental data, including relevant mechanistic data, it is concluded that no-effect-levels exist for both carcinogen-induced precursor effects and neoplasia. Accordingly, we conclude that, at non-toxic dosages, thresholds exist for the induction of experimental cancer by all types of carcinogens.

1. Introduction

The experimental induction of cancer by chemicals has long been established to be a complex multistep process [1–3] as depicted in Fig. 1. This in itself suggests that the overall process could have thresholds, since each of the well understood steps in the sequence of obligatory events is subject to a threshold [4]. The concept of thresholds for chemical carcinogenesis has long been deliberated by numerous scientists [5–19]. The dominant paradigm for dose-effect relationships of chemical carcinogens, which was endorsed by National Academy of Science [20], using concepts derived mainly from radiation mutagenicity research, has been that of a linear no threshold (LNT) model. Flaws in this model have been discussed by Calabrese [21]. Concerning the carcinogenic effects of chemicals, the LNT model is based on little data and does not take into account the complexity of the process, especially the different mechanisms of action through which chemical carcinogens are now known to operate. Specifically, it is generally recognized that chemical carcinogens include two broad types which differ in their fundamental properties [12,22,23]. One type of carcinogen is comprised of chemicals that have as their carcinogenic primary mechanism of action the production of nuclear DNA damage, which can lead to irreversible changes in the genome of affected cells [24,25]. Such carcinogens have been designated as genotoxic [24,26] or DNA-reactive [22]. The second type comprises epigenetic [22] or non-genotoxic compounds that lack the property of DNA reactivity, and are tumorigenic through production of other cellular effects, especially those leading to cytotoxicity-induced increased cellular proliferation [22,23,27–30]. Dose-effect carcinogenicity studies bearing on thresholds were initially undertaken with DNA-reactive carcinogens, as a consequence of the fact that, because of their potency, these were the first to be identified and came into use as model carcinogens.

1.1. DNA-reactive carcinogens

DNA-reactive or genotoxic carcinogens operate primarily through a mechanism involving formation of a reactive electrophile of the

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Abbreviations

α-BHC	α-benzene hexachloride
AAF	2-acetylaminofluorene
AFB	aflatoxin B_1
AhR	aromatic hydrocarbon receptor
AN	acrylonitrile
BaP	benzo[a]pyrene
BHA	butylated hydroxyanisole
CD	cumulative dose
CHL	chloroform
d	daily dose
DBP	dibenzo[a]pyrene
DENA	diethylnitrosamine
DMAB	4-dimethylaminoazobenzene
DMAS	4-dimethylaminostilbene
DMNA	dimethylnitrosamine
EB	ethylbenzene
ENU	N-ethyl-N-nitrosourea
ENUR	N-ethyl-N-nitrosourethane
HAF	hepatocellular altered foci
k	constant
LNT	linear no-threshold
MDAB	3′-methyl-4-(dimethylamino)azobenzene
MelQx	2-amino-3,8-dimethylimidazo[4,5-*f*] quinoxaline
MNNG	*N*-methyl-*N*′-nitro-*N*— nitrosoguanidine
NM	*N*-nitrosomorpholine
NOEL	no-observed-effect-levels
PB	phenobarbital
PBO	piperonyl butoxide
PCDD	polychlorinated dibenzodioxins
RID	riddelliine
ROS	reactive oxygen species
TCDD	tetrachlorodibenzo-p-dioxin
VC	vinyl chloride

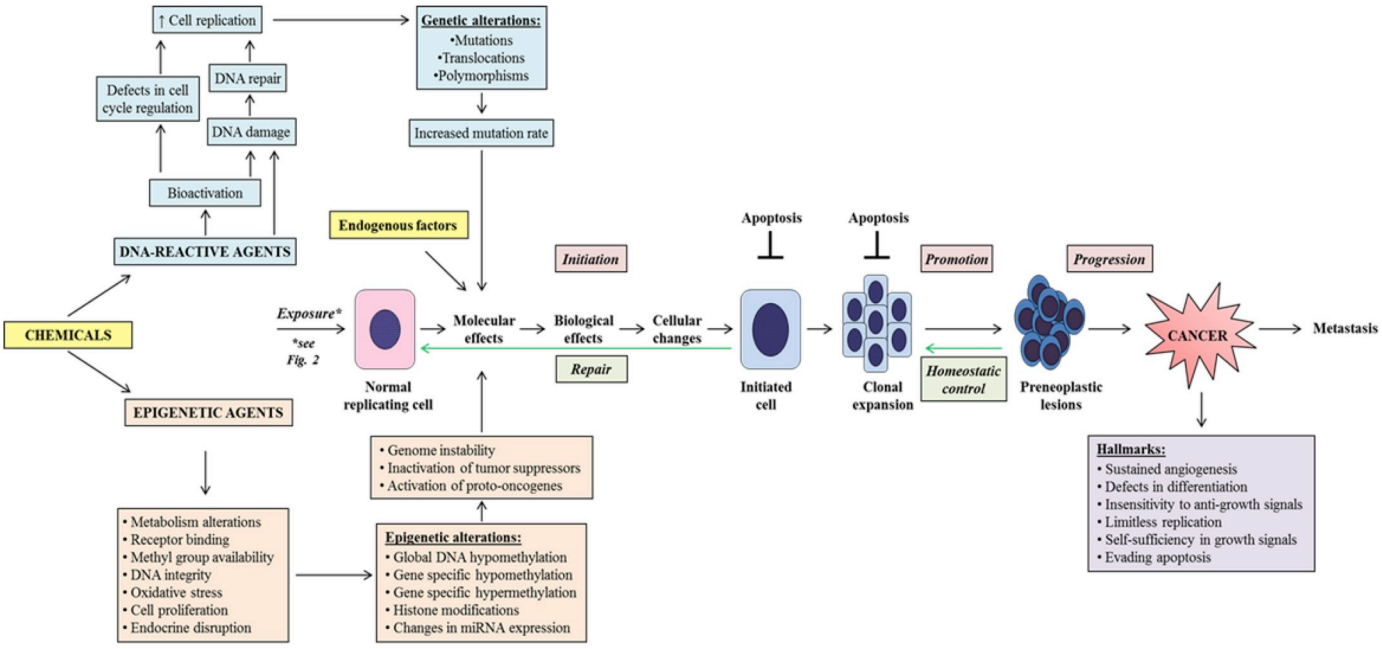

Fig. 1. DNA-reactive and epigenetic triggers and mechanisms of experimental carcinogenesis.

carcinogen (Fig. 2), either through chemical transformation of the parent molecule or through its bioactivation by cellular enzymes in critical (replicating) cells. These electrophiles engage in covalent reactions with nucleophilic cellular macromolecules, most importantly genomic DNA, in critical cells to form chemical-specific adducts or other DNA lesions [32–34]. DNA lesions can be converted to mutations following cell replication. This is reflected by a greater sensitivity of actively proliferating cell cultures to chemical-induced cell transformation [35] and mutagenicity [36]. Specifically, DNA damage incurred during the S-phase of DNA synthesis is highly pro-mutagenic [37] (Fig. 1). Such studies provide mechanistic understanding for *in vivo* findings that, for example, induced cell proliferation in the liver increases its susceptibility to carcinogenicity by DNA-reactive chemicals [38–40]. In genes that regulate cell replication [41,42], such as *KRAS* [43] and p53 [44], mutations can lead to loss of growth control and inception of neoplasia.

DNA-reactive carcinogens can additionally undergo covalent reactions with nucleophilic sites in other cellular macromolecules, particularly at higher doses than those required for DNA adduct formation. Such reactions have been termed epigenetic and suggested to contribute to carcinogenicity [33]. Other epigenetic cellular effects not involving electrophilic reactions are discussed below. They include cytotoxicity and regenerative cell proliferation [45], possibly reflecting RNA or protein binding [33], reaction with mitochondrial DNA [46], or alteration of cellular biochemistry due to receptor binding [47,48].

DNA-reactive carcinogens often produce tumors in more than one species and more than one organ, as with 2-acetylaminofluorene [31], affecting tissues where DNA reaction occurs. DNA-reactive carcinogens because of their potential genotoxicity encompass the most potent chemical carcinogens; indeed, some are active at low doses or following

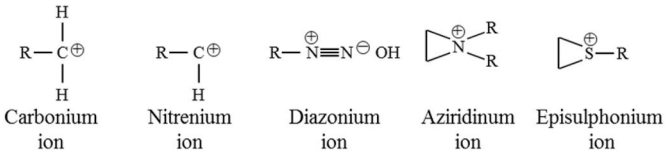

Fig. 2. Reactive electrophiles. Adapted from Kobets and Williams [31].

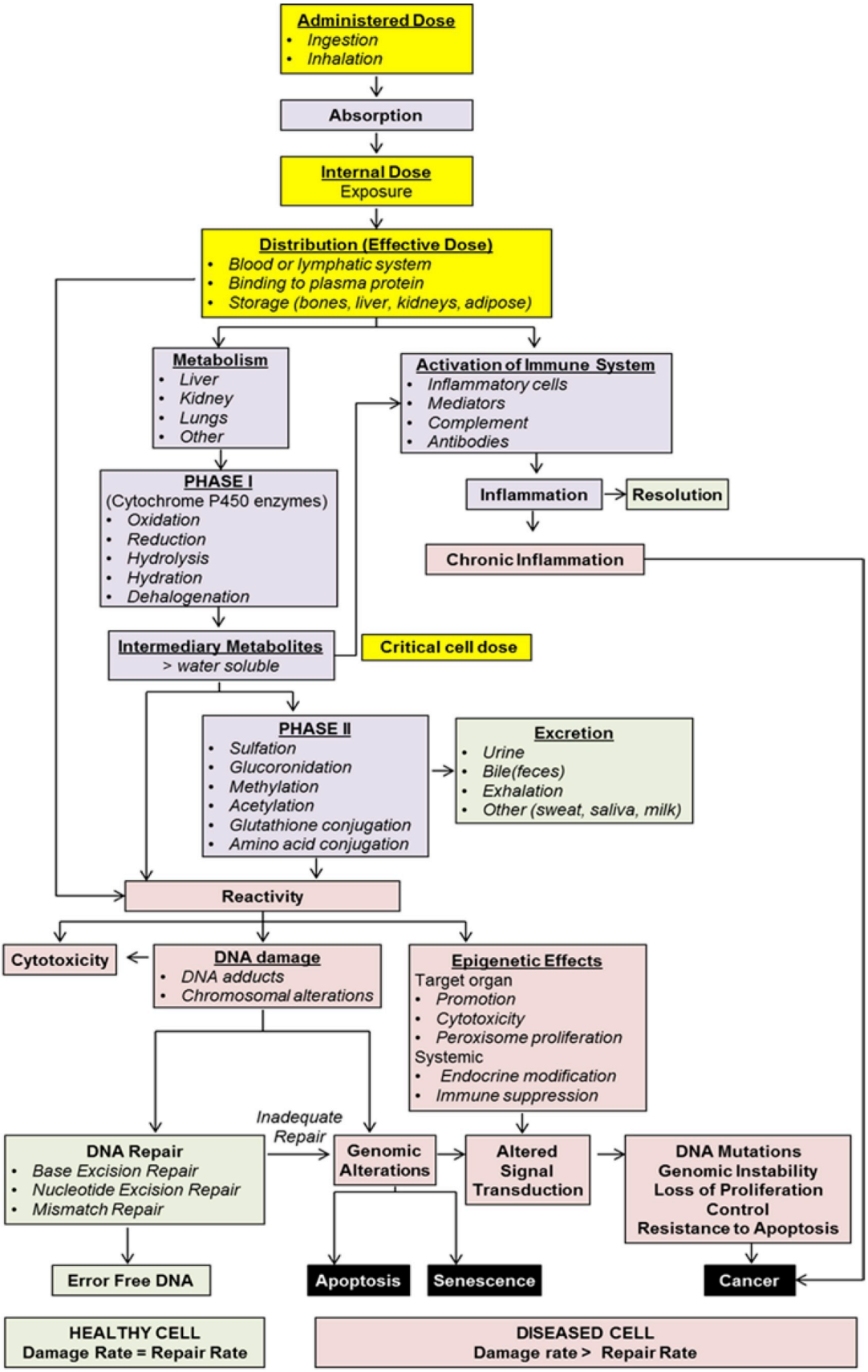

Fig. 3. Chemicokinetics and chemicodynamics of chemical carcinogens.

a single large dose. It has been postulated that DNA-reactivity leading to mutagenicity and carcinogenicity could result from a single direct chemical reaction, specifically, a single hit at a critical site in the DNA of a single target cell. Under these circumstances, threshold responses would not be expected for such DNA-interactive agents, as discussed by Kirsch-Volders et al. [49]. This hypothesis, however, does not take into account the presence in animals and humans of numerous barriers which can protect against the genotoxicity of DNA-reactive carcinogens; these include limited uptake of the carcinogen, enzymatic detoxication, reaction with macromolecules other than DNA, upregulation of adaptation responses, repair of DNA damage, and cellular apoptosis of damaged cells [4,9,50] (Fig. 3), which are discussed in detail below. Accordingly, dose thresholds for cancer induction have been postulated for DNA-reactive carcinogens [4,50–52].

Several dose-effect carcinogenicity studies of DNA-reactive agents have been interpreted as showing no threshold [24,53,54], but none of these have included demonstrably non-toxic doses at the low end of the dose-effect range, as will be reviewed. Specifically, here it is evaluated whether the lowest dosages tested produced toxicities such as DNA adducts formation, which often occurs at doses lower than other adverse effects [55] such as cytotoxicity or histopathological changes. Only dosages without such effects can be regarded as toxicologically "low" dosages and it is these that would be expected to have no-observed-effect-levels (NOEL) for tumors.

Most chemicals that have been judged by the International Agency for Research on Cancer (IARC) to be carcinogenic to humans, are of the DNA-reactive type [56], which, no doubt, reflects their mechanism of action.

1.2. Epigenetic (non-genotoxic) carcinogens

Epigenetic carcinogens, unlike the DNA-reactive type, do not have the chemical structures that lead to formation of reactive electrophiles and accordingly do not engage in covalent reaction with DNA of critical cells [22,57]. Rather, these operate through mechanisms which can result in alterations of tissue homeostasis through a variety of effects on molecular, cellular and tissue components. These effects include alterations in DNA methylation status within gene promoters or globally, histone modifications, receptor binding, cytotoxicity, inhibition of cell-cell communication, oxidative stress and interaction with regulatory systems, notably the endocrine and immune systems. Such effects often lead to increased cell proliferation in target tissues, thereby resulting in tumor increases (Fig. 1) [22,27–30,57,58].

Epigenetic carcinogens usually affect only a single tissue (e.g., butylated hydroxyanisole in the forestomach), unless they produce a systemic effect, such as hormonal modulation. Epigenetic effects produced by carcinogens can also involve molecular and cellular effects that can lead to indirect DNA damage (e.g. oxidative DNA stress and oxidative DNA damage) [59–61]. Some epigenetic carcinogens produce several effects (as with DNA-reactive carcinogens) that contribute to carcinogenicity. For example, hepatocarcinogenic peroxisome proliferator-activated receptor-alpha agonists at carcinogenic dosages not only increase cell proliferation as a result of receptor activation [62], but also induce peroxisome-associated enzymes that lead to generation of reactive oxygen species (ROS) and subsequent DNA oxidative stress [63,64]. Additionally, epigenetic changes to the genome (e.g., alterations in global and gene-specific DNA methylation status, histone modification, miRNA expression) not affecting the DNA sequence, can lead to modification in gene expression and consequent activation of proto-oncogenes and silencing of tumor suppressor genes [65–68].

Epigenetic carcinogens are widely considered to have cancer thresholds [29,52,69–72]. Since epigenetic carcinogens are active only at dosages that produce sufficient biochemical or cellular effects of the type that underlies their carcinogenicity, they are not effective at low non-toxic dosages. Indeed, quite high dosages and prolonged exposure are usually required for their carcinogenicity. Moreover, some carcinogenic epigenetic effects appear to be species specific e.g. male rat renal toxicity produced by α2u-globulin nephropathy inducers leading to kidney neoplasia [73], and induction of rodent liver cell proliferation mediated by peroxisome proliferator-activated receptor-alpha agonism leading to liver neoplasia [74].

Of the many epigenetic carcinogens to which humans are exposed, only a few have been associated with increased risk of cancer. These include hormonal agents and immunosuppressants, for which human intakes resulted in the biologic effect underlying their rodent carcinogenicity [75].

1.3. Thresholds

An important aspect in the elucidation of thresholds for cancer induction is that studies measuring only induction of tumors are limited in their ability to provide evidence of a tumor NOEL. This is because most such studies do not incorporate the very large numbers of animals required to establish statistically the absence of a potential tumor increase at low dosages even though none occurs. Also, most studies typically quantify types of neoplasms which often occur as background pathology and increase with age, thereby complicating comparison between control and dosed groups. In the study of thresholds for tumorigenicity, a frequently applied strategy involves measurements of bioindicators of key toxic effects (see Dose-effect Initiation Studies). These include DNA-adduct formation, cytotoxicity, enhanced cell proliferation, and induction of preneoplastic lesions [45,76–79]. The absence of production of such obligatory precursor molecular and cellular events would preclude tumor induction and hence would constitute a sub-threshold condition. In the present review, if precursor events are elicited at or below the lowest tumorigenic dosage, the experiment is not considered to have tested a "toxicologically" low dose.

In the analysis described here, a tumor threshold for animal carcinogenicity is considered to be a cumulative dose (CD) above which there is induction of neoplasia and below which there is a NOEL for the tumors produced at higher dosages, i.e. a subthreshold dose. A threshold can, of course, be only approximated, as no experiment can achieve precision of measurement of incidences of tumors or preneoplastic events at dosages differing in only a few molecules. The induction of tumors at doses just above the threshold, reflects the response of the most sensitive animals receiving those doses. Above the threshold, effects can be linear, sublinear or supralinear. The experimental conditions (e.g. dose rate, type of exposure) can, of course, influence the effects produced by identical CD.

2. Dose-effect studies of experimental chemical carcinogenicity

In the chemical carcinogenicity literature, a considerable number of long term robust dose-effect studies have been reported. Most have been done with potent DNA-reactive carcinogens and have focused on types of tumors known to be induced by the carcinogen under study.

Here the results are described for all long term animal dose-effect studies found in the literature that were conducted over substantial ranges of dosages (at least three dosages covering an order of magnitude with the high dose being well-tolerated), using continuous repeat dose administration for a significant portion of the life span of the animal (at least 52 weeks for mice and rats), with observation for at least 2 years. The dose-effect studies reviewed report data that directly bear on the existence of cancer thresholds, i.e. the presence or not of NOELs for tumors and preneoplastic effects. When possible, dose is expressed as CD. The dosages (dose rate) reported were always the *administered* dosages, not the absorbed and systemically available *internal* dose (see Chemical Kinetics below).

In describing dose-effect findings, we have included, where available, information on the toxicity of the test substances over the dose range studied; i.e. effect on body weight gain, survival, production of non-neoplastic effects and production of molecular effects, in order to ascertain whether the low dosages tested were non-toxic.

2.1. DNA-reactive chemical carcinogens

Described in this section are examples of dose-effect studies of carcinogenic chemicals recognized to have DNA reactivity as their principal mechanism, as described in the Introduction. Studies are presented in chronological order to document the steady progress over time in study design and interpretation.

The studies described are not all of the dose-effect studies in the literature. For example, the drug tamoxifen is not included, because in the available dose-effect study the dose range did not cover an order of magnitude (5 mg/kg to 35 mg/kg bw/day) [80], and the low dose, which was tumorigenic, was essentially the same as that (6 mg/kg/day) established to produce DNA adducts [81].

2.1.1. Aromatic amines and dialkylnitrosamines

In pioneering research, originally published in German and later in reviews in English, Druckrey [8,24] studied the dose-effects (the term he used) of several carcinogens in rats. These papers are of substantial historical importance, but from the perspective of current testing standards, they have several reporting deficiencies, e.g. the sex of the rats, details of necropsy and incidences of tumors are not given.

The carcinogens studied included the synthetic aromatic aminoazo compound, *4-dimethylaminoazobenzol* or *4-dimethylaminoazobenzene* (DMAB, butter yellow), and *4-dimethylaminostilbene* (DMAS) and several synthetic dialkylnitrosamines, including *diethylnitrosamine* (DENA), all of which were subsequently recognized as DNA-reactive following biotransformation [82,83].

The first experiment [84] used the aromatic amine DMAB administered at doses of 0.1, 0.3, 1.0, 10 and 30 mg daily in the diet to groups of 100 rats starting at 100 days of age and continuing for over 700 days, up to 1000 days for some rats. A control group of 100 rats received no test substance. Complete necropsies were performed and all macroscopic lesions were studied microscopically. The highest dosage induced liver tumors by 35–40 days, whereas the latent period for tumors in the lowest tumor-producing dosage, 1.0 mg/day, was 700 days. The two lowest doses, 0.1 and 0.3 mg, induced no liver tumors, and none were found in controls.

In another experiment [85], DMAB was administered daily as gavage doses of 0.1, 0.3, 1, 3, 10, 20 and 30 mg to groups of rats consisting, respectively, of 158, 148, 169, 70, 30, 15 and 30 rats, starting at 100 days of age. The control group of 120 rats received no gavage. Complete necropsies were performed and all macroscopic lesions were studied microscopically. All rats from the high dose group (30 mg) died before day 32 of dosing. In other dose groups, rats were observed for up to 877 days. At different time intervals several rats from each dose group were terminated and necropsied and their livers were examined microscopically. When a rat died at an advanced age (> 700 days), rats from all groups, including control were necropsied and all tissues with macroscopic changes and the livers were studied microscopically. The median life expectancy for control groups and rats dosed with up to 3 mg/day was 500 days, while for rats that received 10 and 20 mg/day, it was 86 and 52 days, respectively. The high dose (30 mg) group developed liver tumors by 32 days. In other dose groups tumor induction time was 717 days for the 1 mg/day group, 363 days for the 3 mg/day group, 102 days for the 10 mg/day group and 59 days for the 20 mg/day group. The control, 0.1, and 0.3 mg/day groups displayed no tumors.

The aminostilbene derivative DMAS [86] was fed in the diet to seven groups of 400 rats (3 months old) to achieve daily doses of 0.1–3.4 mg/kg. Complete necropsies were performed and all macroscopic lesions were studied microscopically. The time for development of squamous cell carcinomas of the sebaceous ear duct was longer for lower CDs, reaching 900 days at the lowest dosage at which the incidence of carcinomas was 9 out of 12. Non-dosed controls did not develop this tumor.

The alkylating agent DENA [87] was administered in drinking water to nine groups of 16 rats at dosages ranging from 0.07 to 14.2 mg/kg body weight per day starting at approximately 100 days of age and continuing for lifetime. All animals were necropsied and all macroscopic lesions and liver were examined macroscopically. Rats in all dosage groups developed liver tumors, which at the lowest dose was 5 out of 7. Kidney and esophagus tumors were also present. Non-dosed controls were not mentioned. Again, there was no indication of the existence of a subthreshold dose. A larger dose-effect study with DENA [13,88] is described below.

From these experiments, Druckrey [8] concluded that carcinogenicity stemming from continuous dosing in animal experimentation corresponds to dose-effect and time relationships which could be represented by the general formula: dose (d) x time $(t)^n$ = constant ($dt^n = k$, with $n > 1$). He concluded that there was no evidence of a subthreshold dose, although, at low doses tumors developed only after 900 days of dosing and that given the life span of about 1000 days for the rats used, lower dosages would not elicit the manifestation of tumors.

2.1.2. Aflatoxin B_1

Aflatoxin B_1 (AFB) is one of a group of fungal mycotoxins [89]. It can be bioactivated to a DNA-reactive metabolite and is a potent carcinogen [89,90]. It produces primarily liver tumors in rats and some other species, but not in mice [31,89,91], due to metabolic differences [92]. It is classified as a human carcinogen, producing increases in liver cancer, especially in association with hepatitis [89].

Wogan et al. [54] reported a dose-effect carcinogenicity study in which groups of 18–28 male rats were fed five doses between 1 and 100 ppb in a semi-synthetic diet for up to 105 weeks. Necropsies were performed on all animals and microscopic findings in the liver were reported. Hepatocellular carcinomas were induced in high incidence by 50 and 100 ppb, and in lower incidences by lower levels. The high dose group developed a 100% incidence of liver carcinomas by 54–88 weeks, which was substantially earlier than tumor development in the 50 ppb group. The low dose group, consisting of 22 rats developed only 2 carcinomas at 104 weeks, compared to 0 in the control group of 18 rats, which survived to the maximum period. Preneoplastic liver pathology was found in all dosed groups and in 1 control. The authors concluded that the precise character of the dose-response curve could not be inferred from their data.

Subsequently, Buss et al. [93] reported that an average AFB dose level of 2.2 ng/kg body weight per day produced 0.91 AFB DNA adducts per 10^9 nucleotides. The low dose of 1 ppb in the carcinogenicity study is equivalent to 50 mg/kg body weight per day and hence, based on the adduct data, is not devoid of toxicity.

2.1.3. 2-Acetylaminofluorene

2-Acetylaminofluorene (AAF) is a synthetic heterocyclic aromatic amine which following biotransformation is genotoxic and carcinogenic in rodents, primarily in liver, bladder and mammary gland [31,94]. The National Center for Toxicological Research (NCTR) performed a large dose-effect study of AAF designed to estimate the effective dosage producing a 1.0% tumor incidence in mice (i.e. the ED_{01}) [95,96]. A total of 24,346 female mice from a breeding facility were randomly allocated to experimental groups at the rate of 576 per week, requiring 42 weeks to populate the study. Seven concentrations over a 5-fold range of 30–150 ppm in the diet were fed for several intervals of 9–33 months to groups ranging between 144 and 1728 mice, with the higher numbers being allocated to groups receiving lower dosages. At 150 ppm a reduction in survival time of about 10% occurred, which was not entirely attributable to the development of tumors [97]. Necropsies were performed on all animals and extensive microscopic evaluation was conducted [98]. Dose-related increases in liver and urinary bladder tumors were produced [99]. In controls, liver tumors were first found at 14 months and bladder tumors at 9 months. Liver tumors increased substantially in older mice, but bladder tumors did not. Thus, AAF

appeared to be a late acting carcinogen in the liver, whereas it was an early acting carcinogen in the bladder. The dose-effect relationship for the liver tumors appeared to be linear, whereas that for bladder tumors was markedly sublinear at the lower dosages. The incidences of liver tumors at 24 months were 2.3% in the control group and 6.1%, 8.6%, and 12.8% in the three groups fed the lowest concentrations, 30, 35 and 45 ppm, respectively. At this time-point, the incidences of bladder tumors were 0.3% in control group and 1.1%, 0.3% and 0.2% in the three groups fed the lowest concentrations. The investigators, concluded that the data for bladder neoplasms did not contradict the "no threshold" theory of carcinogenesis, while the liver data strongly supported it [96]. The dose range, however, did not cover an order of magnitude.

In a mechanistic follow-up study, Poirier et al. [100] measured formation of DNA adducts in liver and bladder of female mice administered AAF at the dosages used in the ED_{01} study. The major adduct was the deacetylated N-(deoxyguanosin-8yl)-2-aminofluorene adduct. After 28 days of dosing, adducts were present at the dose of 5 mg/kg, which is below the lowest dosage in the ED_{01} study. The adduct levels were linearly related to tumorigenesis outcome in the carcinogenicity study in liver but not the bladder. The authors suggested that in mice administered AAF, in the liver only one AAF-related event, i.e. adducts, is required, whereas in the bladder multiple events are necessary, one of which may be extensive cell proliferation. Thus, in the ED_{01} study the low dosage of AAF was a toxic dose.

2.1.4. Vinyl chloride

Vinyl chloride (VC) or monochloroethane is an industrial gaseous organochloride containing a reactive ethylene double bond. With bioactivation, VC is genotoxic and carcinogenic in experimental animals and humans [75,101]. In humans, it has been implicated in causation of liver hemangiosarcomas and carcinomas [102].

Maltoni et al. [103] conducted five experiments including different dosages in which groups of 60–185 male and female rats were administered VC by inhalation at a total of fourteen dosages ranging from 1 to 30,000 ppm, 4 h/day, 5 days/week for 52 weeks. After dosing, animals were maintained for their lifetime. Survival rate was decreased in rats dosed with the highest dose of VC (30,000 ppm). "Full" autopsy was performed on all animals. Increases in hepatic angiosarcomas and Zymbal gland carcinomas were dose-related. These tumors and several others were not increased at the two lower doses although mammary gland tumors were numerically greater, but not dose-related. The authors concluded that at doses of 5 and 1 ppm no increase was found in specifically VC related tumors. The Maltoni group [103] also conducted long-term oral studies in groups of 80–150 male and female rats administered VC in olive oil at 6 dose levels ranging from 0.03 to 50 mg/kg body weight/day by stomach tube 4–5 days/week, for 52 or 59 weeks. After dosing, animals were maintained for their lifetime. No increase of VC related tumors was observed in rats dosed with the lowest dosage, 0.03 mg/kg body weight/day.

2.1.5. Formaldehyde

Formaldehyde or methanal is an aliphatic gaseous pollutant from natural and industrial sources which is genotoxic and with inhalation is carcinogenic, mainly to the upper respiratory tract [104]. In humans, intake is associated with increased risks of nasopharengeal cancer and leukemia [104]. It is also endogenously produced in all living organisms as a metabolic byproduct and consequently is present in almost all cells [105] and is exhaled in the breath in the low ppb range. The mechanism of action of formaldehyde vapor in inducing nasal tumors when administered by inhalation has been extensively documented and involves cytotoxicity-induced regenerative proliferation in nasal tissues with subsequent development of tumors. While p53 mutations have been measured in nasal tissues following exposure to sufficient doses, they are a late event and not involved in the carcinogenic process [104,106,107]. Genotoxicity and cytotoxicity of formaldehyde may be a consequence of DNA-protein crosslinking [104].

Kerns et al. [108] dosed groups of 119–121 rats and mice of both sexes with 2, 5.6 and 14.3 ppm formaldehyde vapors 6 h/day, 5 days/week for 24 months followed by 6 months of observation. In rats, the mid and high dose groups of both sexes displayed reduced body weight gain. No consistent weight gain changes occurred in mice. High dose male and female rats exhibited increased mortality as did males in the mid dose group. Pathological examinations were performed on all animals. All major tissues from each organ system (approximately 50 tissues per animal) in the control and high dose groups were evaluated microscopically. Tissue masses observed at necropsy were also evaluated microscopically. Squamous cell carcinomas of the nasal epithelium were found in rats of the 6 and 15 ppm groups and mice of the 15 ppm group, but not in lower dose groups. Formaldehyde-induced non-neoplastic lesions of the nasal cavity were found in rats and mice of all dosed groups. They were more severe in rats than in mice.

Monticello et al. [109] dosed 6 groups of 90–150 male rats with formaldehyde by inhalation at 0, 0.7, 2, 6, 10 or 15 ppm 5 days per week for up to 24 months. Formaldehyde induced nasal cancer in greater than 20% of rats at the two highest doses. At 6 ppm only one rat in 90 developed nasal cancer while in the two lowest groups, no rat developed nasal cancer. The authors concluded that 2 ppm was a NOEL.

Kamata et al. [110] dosed 5 groups of 32 (160 total) male rats by inhalation of gaseous formaldehyde at 0.3, 2 and 15 ppm 6 h/day, 5 days/week for 28 months. In the high dose group, significant decreases in body weight and feed consumption were observed. Absolute and relative liver weights in this group were also decreased. Mortality was observed in all groups including the control group, with the highest rate (88.3%) in the highest dose group. Necropsies were performed on all animals and microscopic evaluation was made of 15 tissues and all macroscopic lesions. Epithelial cell hyperplasia and squamous cell metaplasia were observed in all dosed groups, and significantly increased in the 2 and 15 ppm groups compared to control. Nasal tumors were macroscopically evident in the 15 ppm group from the 14th month and 8 of 32 rats bore such tumors at the 24th month. Microscopic examination revealed both squamous cell papillomas and carcinomas. No nasal tumors were observed in the lower exposure groups (0.3 and 2 ppm groups), although non-proliferative and proliferative lesions were found at all dosages, i.e., there were no toxicologically low doses. Study of gene expression in rat nasal mucosa by microarray analysis revealed that gene expression was not altered at 0.7 ppm formaldehyde [111], which supports the tumor NOEL of 2 ppm.

Approaches to characterizing a threshold dose-effect relationship for formaldehyde have been detailed by Clewell and Andersen [112].

2.1.6. N-ethyl-N-nitrosourea

N-ethyl-N-nitrosurea (ENU) is a synthetic alkylating agent, which is directly reactive, resulting in genotoxicity and carcinogenicity [113]. Maekawa et al. [114] conducted a dose-effect study in which 5 groups of 52 male and female rats were administered ENU in drinking water at four concentrations 0.3, 1.0, 3.0 and 10 ppm for 104 weeks, after which rats were maintained until 111 weeks. Based on water consumption, the mean daily intakes were calculated to be as follows: 0.3 ppm-males 0.006 mg, females 0.0045 mg; 1 ppm-males 0.02 mg, females 0.015 mg; 3 ppm-males 0.06 mg, females 0.045 mg; 10 ppm-males 0.2 mg, females 0.15 mg. Growth rates were not affected. In the groups of both sexes given 10 ppm ENU, all animals died before the end of the study. Moribund or dead animals were necropsied. Tumor masses and all organs or tissues were examined microscopically. The first tumor was detected at 41 weeks. In all male groups, including control, tumors were observed mainly in the testis, mammary gland, hematopoietic organs, lung and endocrine organs (i.e. the pituitary gland, thyroid gland, adrenal gland and pancreas). In all female groups, tumors were common in the uterus, mammary gland, hematopoietic organs and endocrine organs, such as the pituitary and thyroid glands. The most common neoplasm in high dose males was brain glioma which was absent in the control group. The low dose group also had no gliomas. Likewise, in females, brain

gliomas were found in 40% of the high dose group, with none in the control or the two low dose groups. The authors noted the difficulty in identifying thresholds, discussed by Preussmann [14], and calculated a virtually safe dose.

2.1.7. N-nitrosomorpholine

N-nitrosomorpholine (NM) is a synthetic cyclic nitrosamine which is genotoxic and carcinogenic with bioactivation [115]. Lijinsky et al. [53] studied the dose-effect of NM in groups of 24–100 female rats administered nine concentrations in drinking water ranging from 0.07 to 100 mg/L (equivalent to doses of ~0.007–9.3 mg/kg body weight per day) 5 days per week for up to 100 weeks at lower doses and 25 weeks for the highest dose, which yielded total doses of 0.7–250 mg per rat. At the end of dosing, rats were allowed to die or were killed when moribund. Survival of the eight lowest dose groups did not differ from controls. Necropsies were performed on all animals and all lesions, major tissues and organs were examined microscopically. Liver neoplasms were present in all groups, including control. In control and lower dose groups, tumors were mostly benign. The number of hepatocellular neoplasms ranged from 6 per 100 rats in the lowest dose group to 35 in 24 rats in the highest dose group. There was an apparent linear relationship of median time of death versus total dose. In the two low dose groups, the incidences of hepatocellular neoplasms were similar. The difference between the liver tumor number in the lowest dose group (6 in 100), which received a total of 0.7 mg, when tested against the multiplicity in controls (1 in 80) was a one sided probability of 0.10 which was considered to “approach significance”. The authors concluded that the results suggest that this lowest dose group was not a no-effect dose.

2.1.8. N-ethyl-N-nitrosourethane

N-ethyl-N-nitrosourethane (ENUR) is a synthetic direct acting alkylating agent which is genotoxic and carcinogenic [116]. Maekawa et al. [117] studied the dose-effects of ENUR administered to 5 groups of 40 female rats for 2 years at 4 doses in drinking water, ranging from 0.15 to 10 ppm. All animals were necropsied and tumor masses and all organs or tissues were examined microscopically. In the upper digestive tract, the initial site of carcinogen contact, squamous cell papillomas or carcinomas, were induced in the three higher dosage groups, whereas none was found in the lowest dosage group. The authors calculated a virtually safe dose, as was done for ENU.

2.1.9. Dimethylnitrosamine, diethylnitrosamine

Dimethylnitrosamine (DMNA) and *diethylnitrosamine* (DENA) are dialkylnitrosamines which are produced as by-products of several industrial processes and can be found in food and water. With bioactivation, they are converted to alkylating agents which are genotoxic and carcinogenic [82,118]. Peto et al. [13,88] conducted large dose-effect studies in a total of 4080 rats introduced into the study from 10 batches from which rats were randomly assigned to experimental groups. Groups of about 60 male and female rats and 240 controls were administered 16 concentrations of DMNA or DENA in drinking water ranging from 0.033 to 16.896 ppm for intervals up to lifetime. Survival was good in low dose groups, up to about 3.5 years. Animals with “clearly palpable” liver abnormalities were terminated and necropsied. Macroscopically evident lesions were examined microscopically. Only a few sections of apparently normal liver and esophagus were taken routinely.

Four principal carcinogenic effects were found: DENA on the esophagus and liver hepatocytes and DMNA on liver hepatocytes and bile duct cells. DENA produced a high incidence of esophageal tumors (77%) in males at the high dose of 16.896 ppm, but none was found in the 4 lowest dose groups. Similar findings were made for both fatal and incidental esophageal tumors. DENA also produced high incidences of fatal hepatocellular neoplasms in males and females, but none was found in the 4 lowest dose groups of males or in the 2 lowest dose groups of females. DMNA produced high incidences of fatal hepatocellular neoplasms in both males and females. No increase in hepatocyte neoplasms was found in the 4 lowest dose groups of males or in the 2 lowest dose groups of females. Fatal bile duct neoplasms were also increased in males and females. No increase occurred in the eight lowest dose groups of males or in the 6 lowest dose groups of females. Non-neoplastic lesions in target organs were not increased in low dose groups. Despite the observed absences of tumors in some low dose groups, the authors concluded that the induction of liver neoplasms by DENA and DMNA showed no indication of any threshold. In a second paper Peto et al. [13], concluded that no purely mathematical argumentation can yield from the data reliable estimates of the effects of very low doses of the carcinogens studied.

2.1.10. Riddelliine

Riddelliine (RID) is a pyrrolizidine alkaloid produced in plants. It can be biotransformed to form a DNA-reactive metabolite [119,120], and it induces hemangiosarcomas of the liver in mice and rats [102,121]. In a long-term carcinogenicity study, groups of 50 male or female mice were administered RID by gavage at doses of 0 or 3.0 mg/kg body weight per day, 5 days per week, for 105 weeks. Additional groups of 50 male mice received 0.1, 0.3 or 1 mg/kg for 105 weeks [120,122]. All animals were necropsied and all organs and tissues were examined and studied microscopically. Male mice in 3 mg/kg group developed multiple liver hemangiosarcomas in 31/50 mice. No increase was found in male mice from lower dose groups. Female mice in the 3 mg/kg group developed alveolar/bronchiolar adenomas or carcinomas (13/50). Hepatocyte cytomegaly was increased in mice dosed with 0.3 mg/kg and above, but not at 0.1 mg/kg.

Groups of 50 male or female rats received RID by gavage at doses of 0 or 1.0 mg/kg body weight per day, 5 days per week, for either 72 (males) or 105 (females) weeks. Additional groups of 50 female rats received 0.01, 0.033 or 0.1 mg/kg [120]. All animals were necropsied and all organs and tissues were examined and studied microscopically. In males and females in 1 mg/kg groups hemangiosarcomas were present (43/50 males and 38/50 females). In female rats from lower dose groups no increase in the incidences of hemangiosarcomas was observed. Hepatocyte cytomegaly was increased at 0.033 mg/kg and above, but not 0.01 mg/kg, and hepatocyte eosinophilic foci were increased only at the two top doses.

In the livers of female rats which received RID for either 3 or 6 month, DNA adducts were formed in a dose-related manner at all tested doses, including the doses at which no tumors were observed. Analyzing the carcinogenicity findings, Chan et al. [122] concluded that the incidences of induced non-neoplastic lesions indicated that the lowest dose levels were close to NOEL and that RID under the experimental conditions employed demonstrated a dose-response relationship in male mice and female rats.

2.1.11. 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline

2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline ($MeIQ_x$) is a heterocyclic amine formed as a product of food pyrolysis. With bioactivation it is genotoxic and carcinogenic [91,123]. Murai et al. [124] studied the dose-effect of $MeIQ_x$ in groups of 51 male rats fed 0.001, 1 or 100 ppm in the diet. Survival of the high dose group was reduced. Necropsies were performed on all rats. Livers were examined and nodules/masses in other organs in which tumors are reported to be induced by $MeIQ_x$ were recorded. All lesions were examined microscopically. The authors reported increased incidences of hepatocellular carcinomas, adenomas, and preneoplastic foci, and increased levels of adducts at 100 ppm. With 0.001 and 1 ppm no significant inductions of hepatocellular preneoplastic or neoplastic lesions were evident, consistent with no significant increase in DNA adducts at 1 ppm. No increase in liver 8-hydroxy-2′-deoxyguanosine levels were found. These findings were considered by the authors to provide evidence for the existence of a threshold, at least a practical threshold, for the carcinogenicity of MeIQx.

2.1.12. Benzo[a]pyrene

Benzo[a]pyrene (BaP) is a non-heterocyclic polycyclic aromatic hydrocarbon which is a product of organic combustion [125]. With bioactivation, it forms a diolepoxide which is genotoxic and carcinogenic [125]. In humans, BaP was judged by IARC [125] to be carcinogenic; in combination with other polycyclic aromatic hydrocarbons it is associated with increased incidences of skin and lung cancers. Wester et al. [126] conducted a dose-effect study in groups of 62 rats of both sexes in which BaP was administered by gavage 5 days per week at dosages of 3, 10 or 30 mg/kg body weight per day for 2 years. Survival and body weight were not reduced in the low dose group. Necropsies were performed on all animals. More than 30 tissues and all macroscopic lesions were examined microscopically. The main tumors induced were hepatocellular carcinomas and forestomach tumors. At the lowest dosage, small incidences of liver tumors were induced in 4/52 males and in 2/52 females, compared to 49/52 and 39/52, respectively, in the highest dosage group. In controls, no liver tumors were found. The liver tumors in the two highest dose groups were mainly carcinomas. Of the liver tumors in low dose males 3 out of 4 were benign and in females all were benign. Non-neoplastic lesions in the liver and forestomach were present in the low dose group. Also, some other neoplasms which were present at high incidences in the high dosage groups were absent in the low dosage groups, i.e., oral cavity neoplasms. The authors did not comment on the possibility of thresholds.

2.2. Epigenetic chemical carcinogens

Described in this section are dose-effect studies of carcinogens for which there is evidence of an epigenetic mechanism of action as described in the Introduction. None of these chemicals has been associated with increased risk of human cancer.

2.2.1. Sodium saccharin

Saccharin (benzoic sulfimide, sodium salt) is a synthetic sweetener. It is not DNA-reactive and its sodium salt operates through a tumor promoting mechanism in the urinary bladder of rodents to induce bladder cancer [127–129].

Several one generation and two generation studies have reported the bladder carcinogenicity of sodium saccharin in male rats, but few involved a wide dose range [130].

Taylor et al. [131] performed a study in which rats of both sexes were dosed starting in utero. Groups of 48 offspring of parents dosed from weaning through mating, gestation and lactation, were fed sodium saccharin at dietary levels of 0.01–7.5%. The study was continued until survival in a group fell to 20% with the last survivors being killed approximately 28 months after the first weanlings were started on the study. All animals were necropsied and 24 tissues were examined microscopically. An increased incidence of urinary bladder hyperplasia occurred in female rats that received 7.5% sodium saccharin, but the lesion was not morphologically precancerous. An increased incidence of urinary bladder neoplasms occurred in the males fed 7.5% sodium saccharin. A total of 11 bladder neoplasms (9 in males, 2 in females) was observed in rats on study longer than 18 months; 9 in the 7.5% saccharin group and 1 each in the control and 5.0% saccharin groups. No neoplasms occurred in the 0.01 or 1% groups.

2.2.2. Amitrole (3-amino-1,2,4-triazole)

Amitrole is a synthetic aminotriazole herbicide [132]. It is not DNA-reactive, but induced thyroid tumors, mainly in rats [132,133] as a result of effects on thyroid function [133].

Steinhoff et al. [133] conducted a dose-effect study in groups of 75 male and female rats aged 6 weeks administered amitrole in the diet at concentrations of 0, 1, 10 and 100 μg/g of feed until death or termination due to morbidity. The average survival was over 900 days. Necropsies were performed on all animals. Tumors and suspected neoplasms as well as 25 organs were examined microscopically. The control group developed 5 benign and 3 malignant thyroid tumors in males and 7 benign tumors in females. There was a marked increase in thyroid tumors in the high-dose groups in both sexes. In low dose groups, the numbers of benign and malignant thyroid tumors were 9 and 0, respectively in males and 12 and 1 in females. In the mid-dose groups, the number of benign and malignant thyroid tumors, respectively, were 4 and 3 in males and 8 and 4 in females. These doses were considered not to induce tumors. The authors concluded that a threshold dose exists for amitrole carcinogenicity. Consistent with this, no tumors were induced in similarly dosed mice or hamsters.

2.2.3. Butylated hydroxyanisole

Butylated hydroxyanisole (BHA) is an antioxidant food additive. IARC [134] concluded that there was sufficient evidence for the carcinogenicity of BHA to experimental animals, based on induction of forestomach tumors in rats and hamsters. BHA is not DNA-reactive and exerts a tumor promoting effect in the squamous forestomach of rodents [72,135] leading to development of squamous cell carcinoma.

Ito et al. [136,137] reported that feeding BHA at 20,000 ppm in the diet for two years induced squamous cell neoplasms of the rat forestomach whereas NOELs for forestomach hyperplasia, papillomas and carcinomas were found at 1250, 5000 and 10,000 ppm.

Whysner et al. [138] reported a life time study of tumor promotion by BHA in the rat forestomach. Groups of 27 male rats were gavaged with a single dose of the forestomach cancer initiating agent *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), and after 3 weeks on control diet, six groups were fed diets containing 60–12000 ppm BHA until termination of the experiment at approximately 110 weeks, at which time most animals had died with stomach tumors. All animals received complete necropsies. Stomachs were prepared for complete visualization of both nonglandular and glandular portions. In addition to stomach, tumors in other organs were studied microscopically. MNNG alone caused a high incidence of tumors in the glandular stomach and forestomach. Feeding of 12000 and 6000 ppm BHA, caused significant increases in the time-related incidences of MNNG-induced forestomach tumors, as analyzed by life table analysis, whereas 3000 ppm or lower doses did not.

2.2.4. Acrylonitrile (vinyl cyanide)

Acrylonitrile (AN) is a chemical monomer used in the manufacture of plastics, rubber and nylon and acrylic fibers [139]. It forms a reactive metabolite, cyanoethylene oxide, and has exhibited some genotoxicity [139], but conclusive evidence for DNA reactivity has not been found [140,141]. Alternatively, induction of oxidative DNA damage has been reported in rat brain [141,142] a target tissue for AN carcinogenicity [139] and in cultured rat glial cells [143]. A dose-effect study was conducted by Johannsen and Levinskas [144] in groups of 100 male or female rats administered dose levels of 1, 3, 10, 30 and 100 ppm in drinking water for approximately 2 years (females 24 months and males 26 months). All animals underwent a full necropsy. Approximately 40 tissues and organs were collected from all animals for microscopic examination. Increases were observed in brain astrocytomas, ear canal neoplasms, and forestomach squamous cell neoplasms in both sexes. The incidences of astrocytomas were greater than 20% at 100 ppm, whereas no increase occurred at 10 ppm or lower. The incidences of ear canal neoplasms were greater than 5% at 30 and 100 ppm with no increase at 3 or 1 ppm. No increase in forestomach neoplasms occurred at 1 ppm. The authors did not comment on dose-effect relationships, but clearly 1 ppm was a tumor NOEL for all three target tissues.

2.2.5. Ethylbenzene

Ethylbenzene (EB) is an industrial chemical and is also used as a solvent in many products [145]. It is not genotoxic in standard assays [146,147]. IARC [145] concluded that there was sufficient evidence of carcinogenicity based on increased kidney tumors in male rats, increased lung adenomas in male mice and increased liver adenomas in female mice.

Chan et al. [148] and NTP [147] reported a bioassay in which groups of 50 male or female rats were administered 0, 75, 250 or 750 ppm of EB by inhalation for 6 h per day, 5 days per week for 104 weeks. Survival was decreased in the high dose groups. All animals were necropsied and visible lesions noted. All organs and tissues were examined microscopically. In male rats exposed to 750 ppm, the incidences of renal tubule adenoma and adenoma or carcinoma (combined) were significantly greater than the chamber control incidence, i.e. 21 out of 50 versus 3 out of 50, whereas the incidences in the low dose group, 5 out of 50, and the mid dose group, 8 out of 50, were not increased. In addition, renal tubular hyperplasia occurred in 10/50 controls, 7/50 low dose, 9/50 mid dose and 17/50 high dose. Only the high dose group was significantly increased. At the high dose, the severity of nephropathy was increased, suggesting that cytotoxicity to the kidney could be the mode of action for EB carcinogenicity. Likewise, in females the severity of nephrotoxicity and incidences of renal tubule hyperplasia and adenomas were increased only in the high dose group. While the authors did not comment on NOEL, the low and mid doses did not exhibit an increase in kidney preneoplasia or neoplasia.

2.2.6. Chloroform

Chloroform (CHL) is an environmental contaminant which is carcinogenic in rodents, including mice and rats, producing hepatocellular adenomas and carcinomas in mice, malignant kidney tumors in male rats and tumors of the thyroid in female rats [149]. It is not genotoxic [149]. Yamamoto et al. [150] conducted dose-effect studies in both mice and rats of vapor-air mixtures of CHL. Groups of 50 mice or rats of both sexes, were administered concentrations of 0, 5, 30 or 90 ppm for mice and 0, 10, 30 or 90 ppm for rats for 6 h/day, 5 days per week for 104 weeks.

In mice, survival was not affected by dosing, although body weight gain was reduced. All animals were necropsied, and all organs were examined macroscopically. Sections of all tissues and tumors were examined microscopically. In males, combined hepatocellular adenoma and carcinoma were 14 (in controls), 7, 12, and 17 for low, mid and high dose groups, respectively. The numbers of combined renal cell adenomas and carcinomas were 0, 1, 7, and 12. In females, the numbers of liver tumors were 2, 2, 4 and 6, while no kidney tumors occurred. Thus, the low dose of 5 ppm was a NOEL for tumors in mice.

In rats, survival was not affected in females, whereas it was reduced in males. Growth rates in both high dose groups were reduced. No statistically significant increase either in liver or kidney tumors was found.

Microscopic changes were found in mouse kidneys in males at 30 and 90 ppm and in rats at all doses. The authors concluded that NOEL for microscopic changes in the kidneys were 5 ppm in mice and 10 ppm in rats.

Golden et al. [151] discuss the mode of action of CHL. They concluded that in experimental animals, CHL is lacking genotoxic properties, and produces liver and kidney tumors only at high cytotoxic doses which exceed the maximum tolerated dose and induce compensatory cell proliferation.

2.2.7. Dioxin

Polychlorinated dibenzodioxins (PCDD) are contaminants formed as by-products in the manufacture of organochlorides of which there is a variety with different structures [152]. Those that bind to the aromatic hydrocarbon receptor (AhR) are referred to as “dioxin-like compounds”, of which *tetrachlorodibenzo-p-dioxin* (TCDD) is the prototypic and most potent congener. PCDD’S do not have structural features that would lead to a reactive electrophile, and are clearly not DNA-reactive, as no DNA binding, or adducts have been found in rodent tissues [152,153]. Absence of DNA reactivity is supported by negative findings in numerous genotoxicity assays [130 [152,154].

The NTP [154] conducted a carcinogenicity study with TCDD in groups of 81 or 82 female rats administered at 3, 10, 22, 46 or 100 ng/kg body weight in corn oil by gavage 5 days per week, for up to 105 weeks. Survival of dosed groups of rats was similar to that of the vehicle control group. Mean body weights of the 22, 46 and 100 ng/kg groups were less than that of the vehicle control group. Complete necropsies were performed on all rats. All organs and tissues were examined for macroscopic lesions, and all major tissues were studied microscopically. Hepatocellular proliferation was increased in a dose-related fashion in all dosed groups, in 100 ng/kg groups it was 5-fold higher compared to control. Liver weights were significantly increased in all dosed groups. A high incidence of liver tumors was induced by the high dose, but no liver tumors occurred at the lower two doses of 3 and 10 ng/kg body weight.

Pitot et al. [155] studied TCDD liver tumor promotion in female rats initiated with DENA followed by 4 doses of TCDD injected biweekly for 6 months. TCDD at 0.01 and 1.0 μg/kg enhanced the development of preneoplastic liver lesions whereas lower doses of 0.001 and 0.0001 did not. The authors concluded that agents acting exclusively or even predominantly at the tumor promotion stage would be expected to exhibit threshold levels.

In evaluating the health risks of dioxin, the NRC [153] concluded that an adequate scientific basis exists to support the hypothesis that the shape of the relationship between dioxin dose and cancer risk is sublinear at low doses, perhaps reflecting responses indistinguishable from background risk at doses below which dose-response data are available.

3. Analysis of experimental carcinogenicity dose-effect data

In the above summarized long term dose-effect studies, for fourteen DNA-reactive carcinogens, experiments with eight exhibited a NOEL for tumors (i.e. DENA, DMAB, DMNA, ENUR, formaldehyde, MeIQ$_x$, RID, VC), whereas six did not. While DENA did not produce thresholds in the early study [87], more recent and robust studies by Peto et al. [13,88] reported NOELs for the compound. Among the six that did not demonstrate NOELs, the data were suggestive for five (AAF, AFB, DMAS, ENU, NM) (Table 1). Also, as Druckrey [8] noted, although in his studies the lowest dosages elicited neoplasms, the induction time was so long (i.e., 700–900 days) that at lower dosages (which were not tested), tumors would not be manifested within the lifespan of the rats, i.e. there would be a practical threshold. Importantly, all these studies were done using continuous daily dosing. This is likely to saturate processes which are subject to thresholds e.g. DNA repair (see below). The carcinogens that did not exhibit NOELs (AAF, AFB, BaP, DMAS, ENU, NM) were all highly potent DNA-reactive agents and it remains unknown what the tumor effect would be at doses lower than those studied, especially at non-toxic doses, i.e. doses not producing DNA adducts, contrary to the situation with AAF and AFB which produced adducts at dosages below the lowest tested in the tumor dose-effect studies.

The NCTR ED$_{01}$ study of AAF has been the subject of much discussion. The authors of the study concluded that the data for bladder neoplasms did not contradict the “no threshold” theory of carcinogenesis, while the liver data strongly support it [96]. The Society of Toxicology ED$_{01}$ Task Force [156] performed a detailed evaluation of the NCTR study. Their analysis took into account, time-to-tumor, an important factor in quantifying carcinogenic effect, first recognized by Druckrey [8] (see above). This was deemed critical, because there was an apparent tendency for the mice dosed with lower doses of 30–60 ppm to live longer than controls when comparable groups were examined. The authors concluded that when the time-to-tumor distribution seen with AAF is incorporated into risk analysis, the tumor responses were clearly non-linear. Authors from NCTR, Kodell et al. [157], challenged aspects of the report and affirmed the original conclusions.

While the interpretation of the AAF study may be debatable, consideration of the available data on eight other DNA-reactive carcinogens supports the existence of tumor NOELs. Furthermore, additional

Table 1
Dose-effect studies for tumor formation by some chemicals.

Chemical	Animal species	Dose range	Route and duration of intake	Tumor NOEL	References
DNA-Reactive Carcinogens					
2-Acetylamino-fluorene (AAF)	Mice	30–150 ppm	in diet fed for several intervals of 9–33 months	N/D (dose-effect relationship for liver tumors linear, for urinary bladder tumors sublinear)	[95,96]
Aflatoxin B_1 (AFB)	Male rats	1–100 ppb	in semi synthetic diet for 105 weeks	N/D (carcinomas were induced in high incidence by 50 and 100 ppb, and in lower incidences by lower levels)	[54]
2-Amino-3,8-dimethylimidazo [4,5-f] quinoxaline ($MeIQ_x$)	Male rats	0.001–100 ppm	In the diet for 104 weeks	0.001, 1 ppm	[124]
Benzo [a]pyrene (BaP)	Male and female rats	3–30 mg/kg bw/day	By gavage 5 days/week, 104 weeks	N/D (BMDL10 3 mg/kg bw day (hepatocellular carcinoma), 1 mg/kg bw/day (forestomach tumors)	[126]
Diethylnitros-amine (DENA)	Rats	0.07–14.2 mg/kg bw/day	in drinking water for lifetime	N/D (*t* for tumors at lowest dose 900 d)	[87]
	Male and female rats	0.033–16.896 ppm	In drinking water for intervals up to lifetime	0.033, 0.066, 0.0132, 0.264 ppm (esophagus both sexes, liver males) 0.033, 0.066 ppm (liver females)	[13,88]
4-Dimethylamino-azobenzene (DMAB)	Rats	0.1–30 mg/day	In diet for lifetime	0.1, 0.3 mg/day (*t* for tumors at lowest tumor-inducing dose 700 d)	[84]
	Rats	0.1–30 mg/day	By gavage for lifetime	0.1, 0.3 mg/day	[85]
4-Dimethylamino-stilbene (DMAS)	Rats	0.1–3.4 mg/kd/day	In diet for lifetime	N/D (*t* for tumors at lowest dose 900 d)	[86]
Dimethylnitros-amine (DMNA)	Male and female rats	0.033–16.896 ppm	In drinking water for intervals up to lifetime	0.264 ppm (liver males) 0.066 ppm (liver females)	[13,88]
Formaldehyde (methanal)	Male and female mice and rats	2–14.3 ppm	Inhalation 6 h/day, 5 days/week, 24 month	2 ppm rats 2, 5.6 ppm mice	[108]
	Male rats	0.7–15 ppm	Inhalation 6 h/day, 5 days/week, 24 months.	0.7, 2 ppm	[109]
	Male rats	0.3–15 ppm	Inhalation 6 h/day, 5 days/week, 28 month	0.3, 2 ppm	[110]
N-ethyl-N-nitrosurea (ENU)	Male and female rats	0.3–10 ppm	In drinking water for 104 weeks	N/D (no brain gliomas in low dose groups in both sexes)	[114]
N-ethyl-N-nitrosourethane (ENUR)	Female rats	0.15–10 ppm	In drinking water for 2 years	0.15 ppm	[117]
N-nitroso-morpholine (NM)	Female rats	0.7–250 mg	In drinking water 5 days/week for up to 100 weeks	N/D (the difference between liver tumor number in control and low dose (0.7 mg/rat) groups was considered to “approach significance”)	[53]
Riddelliine (RID)	Male mice female rats	0.1–3 mg/kg bw/day (mice) 0.01–1 mg/kg bw/day (rats)	By gavage 5 days/week, 105 weeks	0.1, 0.3, 1 mg/kg bw/day (male mice) 0.01, 0.033, 0.1 mg/kg bw/day (female rats)	[120]
Vinyl chloride (VC)	Male and female rats	1-30,000 ppm	By inhalation, 4 h/day, 5 days/week, 52 weeks	1, 5 ppm	[103]
	Male and female rats	0.03–50 mg/kg bw	By ingestion (stomach tube), 4–5 day/week, 52 or 59 weeks	0.03 mg/kg	[103]
Epigenetic Carcinogens					
Acrylonitrile (AN)	Male and female rats	1–100 ppm	In drinking water for 24 months (females) or 26 moths (males)	1 ppm	[144]
Amitrole	Male and female mice, rats, hamsters	1–100 μg/g	In diet for ~900 days	1, 10 μg/g (mice and rats) 1, 10, 100 μg/g (hamster)	[133]
Butylated hydroxyanisole (BHA)	Rats	1250-20,000 ppm	In diet for 2 years	1250, 2500, 5000, 10,000 ppm	[136,137]
	Male rats	60-12,000 ppm	In diet for 110 weeks, 3 weeks after single administration of N-methyl-N′-nitro-N-nitrosoguanidine	60, 300, 1000, 3000 ppm	[138]
Chloroform (CHL)	Male and female mice and rats	5–90 ppm (mice) 10–90 ppm (rats)	By inhalation exposure 6 h/day, 5 days/week for 104 weeks	5 ppm (mice) 10, 30, 90 ppm (rats)	[150]
Ethylbenzene	Male rats	75–750 ppm	By inhalation exposure 6 h/day, 5 days/week for 103–104 weeks	75, 250 ppm	[147,148]
Sodium saccharin (benzoic sulfimide)	Male and female rats	0.01–7.5%	Parents dosed in utero, offspring fed in diet ~28 months	0.01, 0.1, 1%	[131]
Tetrachlorodibenzo-p-dioxin (TCDD)	Female rats	3–100 ng/kg bw	In corn oil by gavage 5 days/week for up to 105 weeks	3, 10 ng/kg bw	[153]
	Female rats	0.0001–1 μg/kg	Injections biweekly for 6 month, after DENA	0.0001, 0.001 μg/kg	[155]

N/D, not detected; bw, body weight.

data generated on AAF precursor effetcs (see “Initiation” section), supports the existence of thresholds in rat liver.

To further assess thresholds, it would be quite informative to develop data on bioindicators of exposure/effect, such as DNA adducts at dosages below those associated with tumorigenesis. For example, Murai et al. [124], and Williams et al. [55] have reported NOEL for formation of DNA adducts by $MeIQ_x$ and AAF, respectively, which supports the NOELs for tumors for these carcinogens. In a review of carcinogenicity dose-effect relationships, Zeise et al. [158] concluded that non-linearity is common and that auxiliary data should be capable of providing additional information on cancer dose-effect relationships outside the range observable in animal carcinogenicity bioassays. Much progress toward this end has been made in studies of initiating effects of carcinogens, as discussed below.

The dose-effect data on seven epigenetic carcinogens displayed NOELs for tumors (Table 1). This is consistent with the fact that epigenetic carcinogens must produce overt cellular effects (e.g. cytotoxicity) as their principal mechanism of either inducing neoplasia ab initio or eliciting tumor development from background preneoplastic cells [22]. At dosages below those required to produce the critical cellular effects, tumorigenicity does not occur. In particular, several of the epigenetic chemicals reviewed operate through a tumor promoting mechanism (e.g. TCDD, BHA), which itself has been shown to exhibit thresholds [71,138,159], (see below).

In summary, the absence of a threshold for experimental chemical carcinogenesis is not proven by available data.

4. Limited duration dose-effect studies

A considerable number of informative dose-effect experiments have been conducted in which dosing was done for less than the lifetime of the animal. These involve the assessment of either tumor initiation or development, key obligatory events in chemical carcinogenesis (Fig. 1). Initiation can be assessed either by the induction of preneoplastic lesions, which has been demonstrated in several rodent tissues, including skin, liver, colon, kidney, mammary gland, lung and pancreas [160–162], or by tumor development following a maintenance period, either with no further dosing or with administration of a tumor promoting agent to enhance tumor development. Neoplastic development or promotion can be assessed by measuring enhanced development of preneoplastic lesions or tumors resulting from administration of a test substance following initiation [78,155,163].

Numerous dose-effect studies of initiation or promotion have been conducted in rodent liver using preneoplastic hepatocellular altered foci (HAF) as the measure of effect (reviewed by Williams et al.; Kobets and Williams; Fukushima et al. [164–166]). HAF precede the development of neoplasms and have phenotypic and genomic alterations indicative of neoplastic potential [31,76,167–169]. They have higher rates of development into neoplasms than do unaltered normal cells [76,162,169]. Accordingly, induction of liver preneoplastic lesions has been used as a robust bioindicator of the initiation process of carcinogenesis [19,77,78]. Preneoplasia has proven useful for this purpose because it occurs at lower CD and with shorter durations of dosing than that which is needed for tumor induction [163,170]. HAF are detectable in routine histologic sections, but are more readily observed with a variety of histochemical markers such as γ-glutamyltransferase, placental-type glutathione S-transferase, glycogen storage and deficiency in iron storage [162]. HAF can be quantified as the number per unit surface area or volume of liver sections [160].

Dose–effect studies aimed at delineating NOELs for initiation have been conducted with liver carcinogens using preneoplastic lesions, together with other bioindicators of effect, such as DNA adducts, cytotoxicity and increased cell proliferation, as endpoints of dose-effect. The concept underlying the use of preneoplastic lesions is that since they are a prerequisite to neoplasia, a NOEL for induction of preneoplasia will necessarily be a NOEL for neoplasia. This is supported in a series of studies [4,45,79] by the demonstration that administration of carcinogen for 8–16 weeks followed by liver tumor promotion with phenobarbital (PB) yielded liver neoplasms only with CD of carcinogen that produced preneoplastic effects. The sensitivity of an initiation/promotion protocol is evident from the fact that it yields higher tumor incidences at lower CD than occur with chronic administration of the same carcinogen alone [171]. This reflects the acceleration of tumor development by the promoter. Several dose–effect studies in rodent liver using preneoplasia have been reviewed [164–166]. Some of these studies involve carcinogens used in the long term studies discussed above.

Additionally, the same concepts have been applied to study dose-effect for enhancement of tumor development following initiation [155].

4.1. Dose-effect initiation studies

In the studies reviewed here, necropsies were performed on all animals, but microscopic examination was usually limited to established target organs.

A dose–effect study of liver tumor initiation by 3′-methyl-4-(dimethylamino)azobenzene (MDAB), was conducted by Hino and Kitagawa [172] using adenosine triphosphatase deficiency as the marker for HAF. MDAB is a ring methylated derivative of the synthetic aminoazo compound, *N,N*-dimethyl-4-aminoazobenzene, which is genotoxic and hepatocarcinogeninc in rats [173,174]. MDAB was fed to groups of male rats for 24 weeks at seven concentrations ranging from 1 to 300 ppm. The incidences of HAF quantified by adenosine triphosphatase deficiency were dose-related with the highest dose producing a 23-fold increase over control. With the lower doses of 1–20 ppm, the incidences of HAF were below the control background, which the authors suggested could be due to inhibition of background carcinogenesis by MDAB. The data were interpreted to show a “practical threshold” between 20 and 60 ppm.

The genotoxic mycotoxin AFB, which was studied for its chronic dose-effect in liver carcinogenicity [54] (above), was also investigated by Dunaif and Campbell [175] for its dose-effect in liver cancer initiation. Eight groups of male rats were administered AFB_1 by gavage at doses of 40–400 μg/kg daily for 10 doses over a period of 2 weeks following which animals were maintained for 12 weeks. HAF quantified by γ-glutamyltransferase histochemical reaction were induced in a dose-related pattern from 150 μg/kg/day and greater. The two lower dosages, 40 and 100 μg/kg/day induced no foci. The authors concluded that there was a threshold dose-response relationship.

A similar dose–effect study of AFB liver cancer initiation was conducted by the same laboratory [176], using γ-glutamyltransferase as a marker for HAF. AFB was administered to groups of male rats by gavage for 10 days at six daily dose levels ranging from 50 to 350 μg/kg body weight, after which rats were maintained for 12 weeks for development of HAF. The dose–effect relationship for HAF was sublinear, with the highest dose producing over a 1000-fold increase in HAF. The authors concluded that there appeared to be a dosage threshold between about 150 and 250 μg/kg body weight per day. Control rats were not mentioned, but in the previous study [175], no HAF were observed in controls under similar conditions. AFB–guanine adduct levels were directly proportional to dosage after the first dose, but after the 10th dose were much lower in the top three dose groups than after a single dose.

The genotoxic nitrosamine NM, which was the subject of a carcinogenicity dose-effect study by Lijinsky et al. [53] (above), was studied for its dose–effect in liver cancer initiation by Enzmann et al. [177]. Glucose-6-phosphate dehydrogenase, glycogen phosphorylase, and glycogen content measured by periodic acid-Schiff stain were used as markers for HAF. NNM was administered to groups of male rats in the drinking water at five concentrations ranging from 0.006 to 60.0 mg/L for 6 or 12 weeks. The dose–effect curves were nonlinear with a slight positive slope at the low doses and a markedly increased slope at higher

doses. In a double logarithmic plot, the dose-effect did not appear linear and was suggested to reflect a concentration threshold. The apparent nonlinear shape of the dose–effect curves for induction of HAF was hypothesized to suggest that at high doses all mechanisms contribute to carcinogenesis, whereas at low doses the effect was exclusively a function of non-threshold mechanisms. Quantitation of hepatocellular replication by proliferating cell nuclear antigen at up to 6.0 mg/L revealed a dose-dependent increase at 12 weeks, but not at 6 weeks.

Dose–effect studies of liver cancer initiation by $MeIQ_x$, which was studied for carcinogenicity dose-effects (above), were conducted by Fukushima et al. [178] using placental-type glutathione S-transferase as the marker for HAF. $MeIQ_x$ was fed to groups of male rats for 16 weeks at six doses ranging from 0.01 to 100 ppm or at 6 doses ranging from 0.001 to 100 ppm in the diet. No macroscopic lesions were apparent. The numbers of HAF per cm^2 of the rat livers of the four groups administered up to 1 ppm of the carcinogen did not differ from the control values and hence were NOELs. In contrast, a measurable increase in HAF was observed with 10 ppm and a substantial elevation with 100 ppm. At both weeks 4 and 16, linear relationships were found between the various dosages and the levels of MeIQx – DNA adducts measured by nucleotide ^{32}P-postlabeling. In a parallel study in male Big Blue transgenic rats [179], also demonstrated mutagenicity at the *lacI* gene in the liver, at 10 and 100 ppm, but not 1 ppm or lower. The authors concluded that their data provided evidence for NOEL. This supports the evidence for a threshold in the carcinogenicity study.

Fukushima et al. [51] also conducted a longer duration dose–effect study of $MeIQ_x$ initiation using placental-type glutathione S-transferase as a marker for HAF. $MeIQ_x$ was fed to groups of male rats for 32 weeks at six concentrations in the diet ranging from 0.001 to 100 ppm. No macroscopic lesions were apparent. The numbers of HAF per cm^2 of the rat livers of the four groups receiving up to 1 ppm of the carcinogen did not differ from the control value and hence were NOEL. In contrast, an increase was observed with 10 ppm and a substantial elevation with 100 ppm $MeIQ_x$. Thus, at dosages below 10 ppm, $MeIQ_x$ did not induce a measurable increase in HAF, but did form DNA adducts at 0.1 ppm and above. Based on the findings, the authors concluded that NOELs exist for key parameters relevant to carcinogenicity. In a follow-up 2-year carcinogenicity study with MeIQx in male rats using some of the same dosages 0.001, 1 and 100 ppm in the diet (see above), Murai et al. [124] reported increased frequencies of hepatocellular carcinomas, adenomas, and HAF, and increased levels of adducts at 100 ppm. With 0.001 and 1 ppm no significant increases in hepatocellular preneoplastic or neoplastic lesions were evident, consistent with no significant increase in DNA adducts at 1 ppm. The authors concluded that a threshold, at least a practical threshold, exists for MeIQx carcinogenicity.

Fukushima et al. [51] conducted a dose–effect study of liver cancer initiation by *N*-nitrosodiethylamine, referred to here as DENA, which was studied for carcinogenicity dose-effects (above). DENA was administered in the drinking water to groups of male rats for 16 weeks at six concentrations ranging from 0.0001 to 10 ppm. No macroscopic lesions were evident. The numbers of liver HAF identified by placental-type glutathione S-transferase in the three groups that received the lower dosages of DENA, 0.0001, 0.001 and 0.01 ppm, were not different from those of the control, while the groups administered 0.1 or 1 ppm DENA showed significant increases in HAF. The authors concluded that a NOEL may exist for induction of foci.

VC, as indicated above, with bioactivation, is genotoxic and carcinogenic [75,101]. Two studies of liver cancer initiation by VC were conducted by Laib et al. [180] using a protocol which differed from others reviewed here. Groups of newborn male and female rats were dosed by inhalation at 10 doses between 2.5 and 2000 ppm starting on day one after birth. In one study, the dosing was for 10 weeks (8 h/day, 5 days/week), followed by 1 week of recovery before termination, and in the second, dosing was for 3 weeks (8 h/day, 5 days/week), followed by 10 weeks of recovery before termination. Adenosine triphosphatase-deficient HAF were induced in both sexes with a linear relationship between the dose of VC and the percent of induced HAF with curves which run through the origin. It was noted that the mean foci area induced by the two lowest doses in males lies within the rage of foci area of the corresponding controls. Nevertheless, the authors concluded that within the dose range investigated, no obvious threshold for the induction of HAF by VC was observed, in contrast to the chronic studies (above). Assessment of toxicity, however, was not conducted.

In a series of studies using the DNA-reactive hepatocarcinogens DENA and AAF [4,171], which were investigated for carcinogenicity dose-effect (above), evidence of NOEL for these DNA-reactive carcinogens in male rat liver was demonstrated. In these investigations, the effects of the carcinogens were quantified by measurement of DNA adducts, hepatocellular cytotoxicity, cell proliferation (quantified as the proliferating cell nuclear antigen -positive replicating fraction), and formation of placental-type glutathione S-transferase positive HAF, in the initiation phase of carcinogenesis. Also, phenobarbital promotion was used to elicit manifestation of initiation of liver carcinogenicity by the formation of neoplasms after 24 weeks. In these studies, both carcinogens were administered either by injection or gavage to achieve precise dosing on a body weight basis, expressed as CD.

With DENA [79,181,182], the CD of 25.5 mg/kg body weight (the lowest dose tested), administered by intraperitoneal injection over 10 weeks, was a NOEL for cell proliferation. HAF were increased, although they were not promotable to hepatocellular neoplasia (by 24 weeks administration of PB). At this toxicological effect level, about 14 DNA adducts in 10^8 normal nucleotides were formed, and at 51.1 mg/kg body weight, which yielded promotable neoplasia, 200 adducts/10^8 nucleotides were formed. The adducts, which were quantified by HPLC analysis with fluorescence detection, were 7-ethylguanine and O^6-ethylguanine, the latter at a site of base pairing and hence a miscoding lesion [183]. A NOEL for DNA adduct formation was not observed in the dose range studied.

With AAF [45,55,184], the CD of 28 mg/kg body weight, delivered by gavage over 12 weeks, was a NOEL, for both hepatocellular proliferation and HAF, as were three lower CDs. At 28 mg/kg, promotable (with 24 weeks of PB) hepatocellular neoplasia was not produced, indicating absence of initiation. Importantly, at this CD, only about 6 DNA adducts in 10^8 nucleotides were formed, as measured by nucleotide ^{32}P-postlabeling.

Thus, a NOEL for DNA adduct formation by either DEN or AAF was not found in the above studies. Two further dose–effect experiments have been reported at lower repeat doses of AAF [55] than those used in the previous studies. In addition, the specific types of DNA adducts formed were identified. AAF was administered by gavage to male rats at repeat dosages, which in one experiment ranged from 0.01 to 2.24 mg/kg body weight per day, 7 days/week for 12 weeks followed by recovery for 4 weeks, and in a second, at lower dosages of 0.0026 or 0.026 mg/kg body weight per day 3 days per week for 16 weeks. Such prolonged dosing yields a steady-state condition of DNA adduct formation and repair. In the liver, following a single dose, in the nucleotide ^{32}P-postlabeling assay, the non-acetylated guanine adduct, *N*-(deoxyguanosine-8-yl)-aminofluorene, predominated. With continued dosing, the pattern of adducts changed such that by 4 weeks more acetylated adducts, *N*-(deoxyguanosine-N^2-yl)-acetylaminofluorene and N-(deoxyguanosine-8-yl)- acetylaminofluorene, were present. In the first experiment, total adducts reached a maximum by 12 weeks with levels of 6.0 adducts per10^8 nucleotides at the lowest CD. In the second, the total DNA adducts at the lowest CD was below the limit of detection at 12 weeks, and the chromatographic density was consistent with 0.6 in 10^8 nucleotides at 16 weeks, a level within the background range of 1.0–3.1 in 10^8 nucleotides. Thus, the CD of 0.125 mg/kg body weight over 16 weeks was concluded to be a NOEL for adducts.

In these initiation studies, DNA adducts were formed at CD of AAF which were below those that elicited increases in either hepatocellular proliferation or HAF, as found with other carcinogens [51,176,178].

The adduct levels at NOEL for other effects were at or below 1 in 10^9 nucleotides [171], which is considered to be of questionable biologic significance (see Molecular Events and Cellular Reaction in Critical Cells below).

A very large dose-effect study of dibenzo [a]pyrene (DBP), a DNA-reactive aromatic hydrocarbon, was conducted by Bailey et al. [185] using over 32,000 rainbow trout, presumably of both sexes. Fish were exposed to seven doses of DBP ranging from 0.45 to 225 ppm in the diet, for an initiating period of 4 weeks after which they were maintained for a further 9 months. Liver and stomach neoplasms were induced. At the lowest dose administered to 4 groups of 2021–2380 fish, the incidences of liver tumors per fish ranged from 0.00047 to 0.00226, compared to 0.00085 to 0.00195 in 4 groups of 1862–2348 controls. Likewise, at the lowest dose, the incidence of stomach tumors ranged from 0.00136 to 0.00420, compared to 0.00043 to 0.00236 in controls. Thus, a substantial overlap of tumor incidences occurred in groups of controls and low dose fish. Moreover, at lower doses the hepatic tumor response fell below direct proportionality with DBP dose. Two biomarkers of initiation, DNA adducts and cell proliferation, were also studied after 4 weeks of dosing. No effect of DPB was found on cell proliferation in the liver or stomach. Liver DNA adducts were linear with dose and were present at the lowest dose, which was suggested to be due to the deficiency of trout in global excision repair. The authors concluded that "although the data were consistent with a threshold interpretation, even the use of over 30,000 animals did not provide *proof* that a threshold was reached, or would exist, in either target organ." Nevertheless, the low dose produced cellular toxicity in the form of DNA adducts.

4.2. Enhancement of initiation studies

Pitot et al. [155] studied the promoting effects of PB and TCDD in female rat liver when administered after an initiating dose of DENA together with partial hepatectomy. Feeding for 6 months of 0.01% and 0.05% PB greatly enhanced the development of liver foci identified by γ-glutamyltransferase, adenosine triphosphatase, or glucose-6-phosphatase histochemistry, whereas 0.005% had no effect and 0.001% actually decreased the development of foci. In another experiment, TCDD was administered by intramuscular injection for 6 months after initiation. Enhancement of foci was produced by 0.01, 0.001 or 0.0001 μg/kg/day, but not 0.1 μg/kg/day. The authors concluded that the responses to these chemicals exhibited threshold levels.

Masuda et al. [186] performed a study of the enhancing effects of α-benzene hexachloride (α-BHC) on male rat liver foci induced by DENA. α-BHC was fed in the diet at 12 doses ranging from 0.01 to 500 ppm for 6 weeks. Increases in the numbers of foci identified by placental-type glutathione S-transferase were found at 2 ppm and greater, but not at the four lower doses.

Muguruma et al. [187] conducted a study of the enhancement by piperonyl butoxide (PBO) of hepatocarcinogenicity initiated by DENA in male rats. Groups of rats received intraperitoneal injections of DENA followed 2 weeks later by 0%, 0.125%, 0.25%, or 0.5% PBO in the diet in one experiment and in the second experiment by 0%, 0.015%, 0.03%, or 0.06% PBO in diet. After one week on test diets, two-thirds partial hepatectomies were performed to enhance hepatocellular proliferation. Rats were terminated at 8 weeks. The numbers of glutathione S-transferase placental-form positive foci were increased in rats which received 0.25% and 0.5% PBO, but not at lower concentrations. Measurement of microsomal ROS production by a fluorescence method revealed increases at the highest dosage of 0.25% and 0.5% PBO. The authors concluded that 0.25% PBO is the threshold dose that induced ROS-mediated hepatocarcinogenesis.

5. Analysis of dose-effect data from limited duration experiments

Most of the limited duration studies of initiation of rat liver carcinogenesis have revealed NOEL for preneoplasia, with the exception of an inhalation study with VC, where a NOEL was not found at a dosage (2.5 ppm) which is below that (5 ppm) at which a NOEL for VC-related neoplasms was observed in a chronic study [103]. The VC study was the only one of those reviewed which used newborn animals. It is established that newborn animals are extremely responsive to VC-induced liver tumors [103] and that young rats are more susceptible to carcinogenicity by VC than are older ones [188]. This may be due to high levels of cell proliferation in the developing liver, which is known to increase susceptibility to neoplasia [39,40,189]. In this study, parameters of toxicity were not assessed and hence the doses were not established to include any that were devoid of toxicity. In the initiation study of DBP in trout, all dosages produced DNA adducts and thus, a non-toxic dose was not tested. With regard to adducts produced in fish, there is data indicating that the amount of DNA repair in cultured trout cells is less than that in mammalian cells [190] and hence, extrapolation of effects to mammals is uncertain.

Thus, the preponderance of the findings in studies of initiation are not consistent with LNT. Rather they support the interpretation that thresholds exist for initiation of carcinogenicity by DNA-reactive carcinogens and accordingly, there are thresholds for tumorigenicity.

Also, studies of enhancement of tumor development or promotion by chemicals administered after initiation all revealed NOELs. This is in accord with the fact that these chemicals are not genotoxic and must produce toxicity for an extended duration to achieve carcinogenicity.

As discussed in the Introduction, carcinogenesis is a multistep process. The steps in the process are each dose related and have thresholds (Fig. 1), as described here for initiation and promotion. Accordingly, the thresholds for these cancer precursor steps determine thresholds for the overall process, as documented above.

6. Essential steps in carcinogenesis which are subject to thresholds

The tumor NOELs identified in the review of dose-effect studies could be a consequence of either negligible internal exposures at the doses at which there were NOEL or to the operation of protective processes. In the studies reviewed, the lowest dose tested was often greater than 1 μg, and was administered repeatedly. Estimating the theoretical internal exposure arising from a dose of 1 μg to a 25 g mouse, yields an exposure of 2×10^{15} molecules per mouse or 1.3×10^5 molecules per cell (Table 2). Given the substantial theoretical internal exposures that would stem from such a dose, it seems likely that some bodily processes must be operative to attenuate potential carcinogenic effects.

The essential processes or key steps in carcinogenesis, each of which following the intake of the chemical is dependent on the preceding, include chemical absorption and distribution, bioactivation (if required), interaction with target replicating (stem) cells, induction of cell mutation, formation of preneoplastic lesions and progression of preneoplasia to neoplasia (Fig. 1). Each of these steps, as discussed below, has barriers, which must be overcome for the process of neoplasia to be accomplished [34]. These barriers can differ between animals in a group and between different groups of animals.

6.1. Absorption distribution and excretion of carcinogens (chemical kinetics)

The administered dose of a carcinogen is subject to pre-systemic biotransformation by enzymes in the gastro-intestinal tract. For example, aminoazo carcinogens, such as DMAB, can undergo azo reduction yielding metabolites which are non- or weakly carcinogenic compared to the parent compound. The absorption of a portion of the *administered dose* of a xenobiotic yields the *internal dose* available to effect carcinogenicity. Absorption of xenobiotics, including both DNA-reactive and epigenetic carcinogens, is determined by the route of

Table 2
Estimated number of molecules in 1 μg of chemical per one cell of a dosed mouse.

Line Number	Value	Value Identification	Calculations[a]
1	1 μg	Amount of a chemical (NOEL)	
2	300 g/mol	Assumed molecular weight	
3	6.022×10^{23} molecules	Avogadro's number	
4	0.0033 moL/g	Molecules per mass	1/2
5	1,000,000 μg/g	Conversion factor	
6	3.33×10^{-9} mol/μg	Converted molecules per mass	4/5
7	**2×10^{15}** molecules per microgram	Number of molecules per dose of chemical per mouse	6 × 3
8	3.7×10^{13} cells	Number of cells in the human[b]	
9	62 kg	Average weight of an adult human	
10	25 g	Average weight of a mouse	
11	4.03×10^{-4}	Mouse to human weight ratio	10/(9 × 1000)
12	1.49×10^{10} cells	Number of cells in a mouse	8 × 11
13	**1.34×10^{5}** molecules per cell	Number of molecules of a chemical per one mouse cell	7/12

[a] indicates reference to the line number, plain text indicates numerical value.
[b] [191]; [273], not including microbiome.

administration. For example, with skin application, most chemical carcinogens are not effectively absorbed. In fact, direct application of sufficient doses of carcinogens by this route usually results only in skin tumors; for example, topical application to mouse skin of the alkylating agent N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) induces skin neoplasia, but not tumors in remote sites. Inhalation or oral dosing results in both local exposure and following absorption and distribution, systemic exposure. Thus, as a result of direct contact, inhalation dosing of rats with formaldehyde leads to nasal cancer and oral administration to rats of MNNG produces stomach cancer. Similarly, with some epigenetic carcinogens, such as BHA fed in the diet, direct contact with forestomach epithelium leads to carcinogenicity.

The internal dose of absorbed carcinogen is available to be systemically distributed throughout the body by way of blood or lymphatic routes. Distribution to internal organs yields the *effective exposure* in recipient organs. This is the exposure available to produce adverse effects to critical cells. Thus, oral administration of DNA-reactive polycyclic aromatic amines (e.g. AAF) at sufficient doses leads to liver, urinary bladder, and mammary gland tumors, organs to which the carcinogen is distributed and where it is bioactivated. Likewise, with the epigenetic carcinogen sodium saccharin, excretion in the urine results in bladder cancer.

Absorption by any route is not complete and low inhaled or oral doses may not result in significant internal exposures. Moreover, systemic distribution throughout the body and excretion in bile or urine reduces the effective exposure of carcinogen reaching critical cells, compared to that which occurs with direct contact. Thus, chemical kinetics can result in reduction of dose effects to toxicologically insignificant levels, which can underlie thresholds; in other words, failure to achieve an effective exposure of critical cells results in no tumor effect.

6.2. Biotransformation of carcinogens

The effective dose of a carcinogen reaching bodily tissues is subject to biotransformation by tissue cellular enzyme systems (Fig. 3). Phase I oxidative or reductive biotransformation is largely mediated by the cytochrome P450 system, which is present at some level in almost all tissues and is highly expressed in some tissues, notably liver [192,193]. The preponderance of biotransformation of DNA-reactive agents results in detoxication and excretion, thereby reducing the effective dose available to interact with critical cells. For example, Oesch et al. [50] documented the central role of microsomal epoxide hydrolase in the detoxication of genotoxic epoxides. Other potentially protective enzymes include those involved in phase II reactions, glucuronide and sulfate conjugation enzymes and aldo-keto reductase (protective macromolecules are discussed below). The effective cell dose is subjected to biotransformation, yielding the *critical cell dose.* In the case of DNA-reactive agents, biotransformation can lead to some level of formation of reactive species, i.e. electrophiles of which there are five distinct types associated with carcinogenicity (Fig. 2) [57]. For epigenetic agents, biotransformation usually is entirely detoxication. Following absorption, much of chemical biotransformation takes place in the liver, which eliminates a large fraction of orally administered xenobiotics, in first pass metabolism, leading to excretion of metabolites in urine and bile (Fig. 3). Most xenobiotics are rapidly cleared, but lipophilic substances, such as dioxin, can persist.

Thus, biotransformation systems provide a further barrier to carcinogens, reducing the ultimate accessibility of carcinogen to the critical cells, which can, thereby, contribute to thresholds [50].

6.3. Critical cell accessibility

The rodent body is comprised of more than 1×10^{10} cells. The critical cells which are susceptible to neoplastic transformation, however, are a small population of replicating tissue renewal cells or adult stem cells (see Mutation below). These cells are substantially outnumbered in most tissues by non-susceptible post replication cells. Proliferating critical cells generally constitute less than 10% of the tissue population [194], although, in highly proliferating tissues, such as gastrointestinal tract with a high growth fraction, they can represent over 50% [195]. Thus, most of the effective dose of a carcinogen which reaches a potential target tissue engages non-susceptible post replication cells thereby reducing the critical cell exposure. Additionally, cells have transport systems, such as p-glycoprotein, which export xenobiotics from the cell [196]. Moreover, replicating cells are programmed for the functions needed for cell proliferation and do not highly express the enzyme systems involved in chemical biotransformation. At high exposures to carcinogens, toxicity can lead to cell death and compensatory cell proliferation which increases the pool of replicating target cells [197]. Also, life stage plays an important role in tissue susceptibility, as noted above for VC. Generally, carcinogens are most effective when dosing is started early in life [188], which may reflect greater numbers of active stem cells.

6.4. Molecular Events and Cellular Reactions in critical cells

Cells, including those that are potential targets for carcinogenicity, are endowed with cytoprotective molecules, notably glutathione. Reactive metabolites (electrophiles) of DNA-reactive compounds (Fig. 2), either those formed in critical replicating (stem) cells or those reaching these cells after formation elsewhere in the body, as well as epigenetic agents, readily undergo reaction with these molecules in the cytoplasm. These reactions proceed according to the laws of chemical thermodynamics, in which reactions are driven by the concentration of

the reactant at the site of action and its chemical potential [198]. This reduces carcinogen available for entry into the nucleus and diminishes consequent effects on the genome, both genotoxic and epigenetic.

From the fraction of a reactive chemical species which reaches the nucleus, a portion can undergo reactions with nuclear proteins (i.e. histones). Since DNA is surrounded by nucleoproteins, electrophilic reactions preferentially take place with these molecules [199], thereby reducing the fraction that reaches DNA and could produce potentially promutagenic lesions.

Furthermore, not every chemical adduct produced in DNA is potentially miscoding, since some are formed at sites on bases that are not involved in base pairing. Also, not every interaction with DNA involves a susceptible site for mutation in critical genes. Most adducts occur in the non-coding regions of DNA which are substantial, since only about

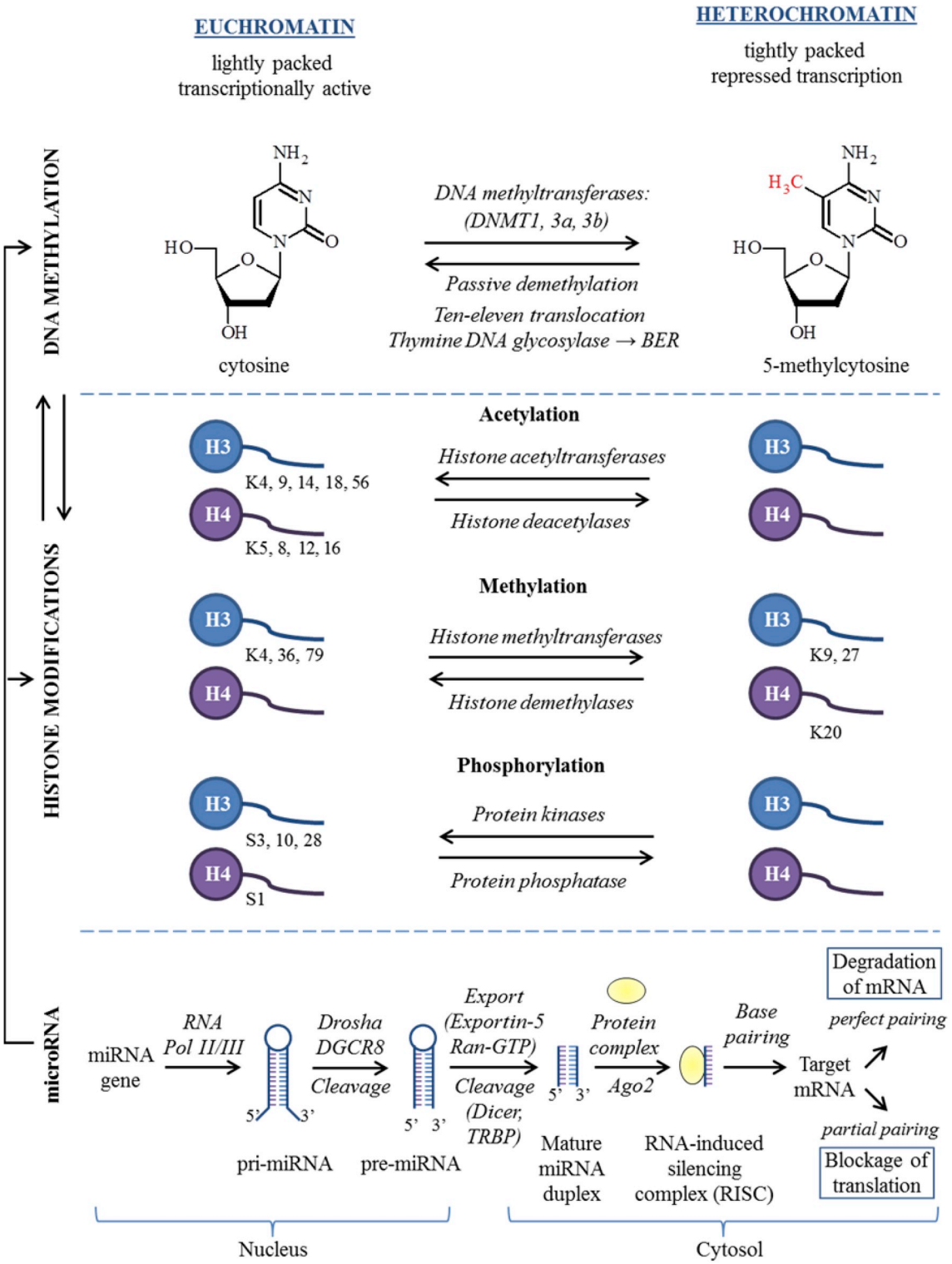

Fig. 4. Molecular epigenetic alterations. BER, Base excision repair; H, histone; K, lysine; miRNA, microRNA (21–30 nucleotides); pri-miRNA, primary miRNA; pre-miRNA, precursor-miRNA; S, serine; RNA Pol, RNA polymerases; TRBP, *trans*-activation response RNA-binding protein.

2% of the genome (exons and their promoters) of somatic cells is potentially transcriptionally active [200,201]. Only lesions in active DNA would be expected to be of biological significance, because they could lead to mutations in genes coding for cellular proteins, and consequently permanently affect cell function.

Importantly, the eukaryotic genome possesses highly efficient and highly error-free DNA repair processes which mitigate effects of DNA reactions (see below). Eukaryotic cells also are equipped with DNA damage response elements (e.g. p53) which are activated by DNA damage [202] and function to slow cell cycle progression [203], thereby reducing susceptibility to mutagenicity. Moreover, in animal tissues, as a result of metabolic processes, genomic DNA has a substantial background of natural-occurring damage [204–207], in the order of 1 in 10^6 nucleotides. Consequently, only a level of carcinogen-mediated DNA damage in excess of this is potentially of biological significance.

DNA-reactive carcinogens can also contribute to cellular transformation through epigenetic effects, such as by causing cytotoxicity and compensatory cell proliferation, as shown for AAF [45]. Doses of DNA-reactive carcinogens below those producing such epigenetic effects display reduced carcinogenicity [45], which could be the basis for thresholds.

Epigenetic carcinogens, by their nature, do not form chemical-reactive metabolites. They can, nevertheless, produce DNA damage through indirect mechanisms such as formation of ROS [59–61,208]. Increase in cellular ROS can result from either increased formation or reduced depletion due to decreased antioxidant levels. ROS actively bind to macromolecules, including protein, lipids, RNA and DNA. Oxidative modifications of DNA bases, for example formation of 8-hydroxy-2′-deoxyguanosine, can lead to mutations and cancer development [59]. The endogenous sources of ROS are intracellular organelles, e.g. peroxisomes, mitochondria and inflammatory cells, e.g., neutrophils [60]. Such processes, however, require a high cellular concentration of the chemical and are inhibited by cellular antioxidant molecules. Moreover, it appears that the cell can accommodate a level of oxidative damage since that is a common background alteration, arising from cellular metabolic processes [61,206,209]. Moreover, oxidative lesions in DNA, are only weakly promutagenic [210,211], which may account for the observation that the carcinogen p-dichlorobenzene, which induces oxidative stress, has activity as a promoting agent, but not an initiating agent [212].

Some epigenetic carcinogens engage in receptor binding which can mediate their effects in a variety of ways. Several epigenetic

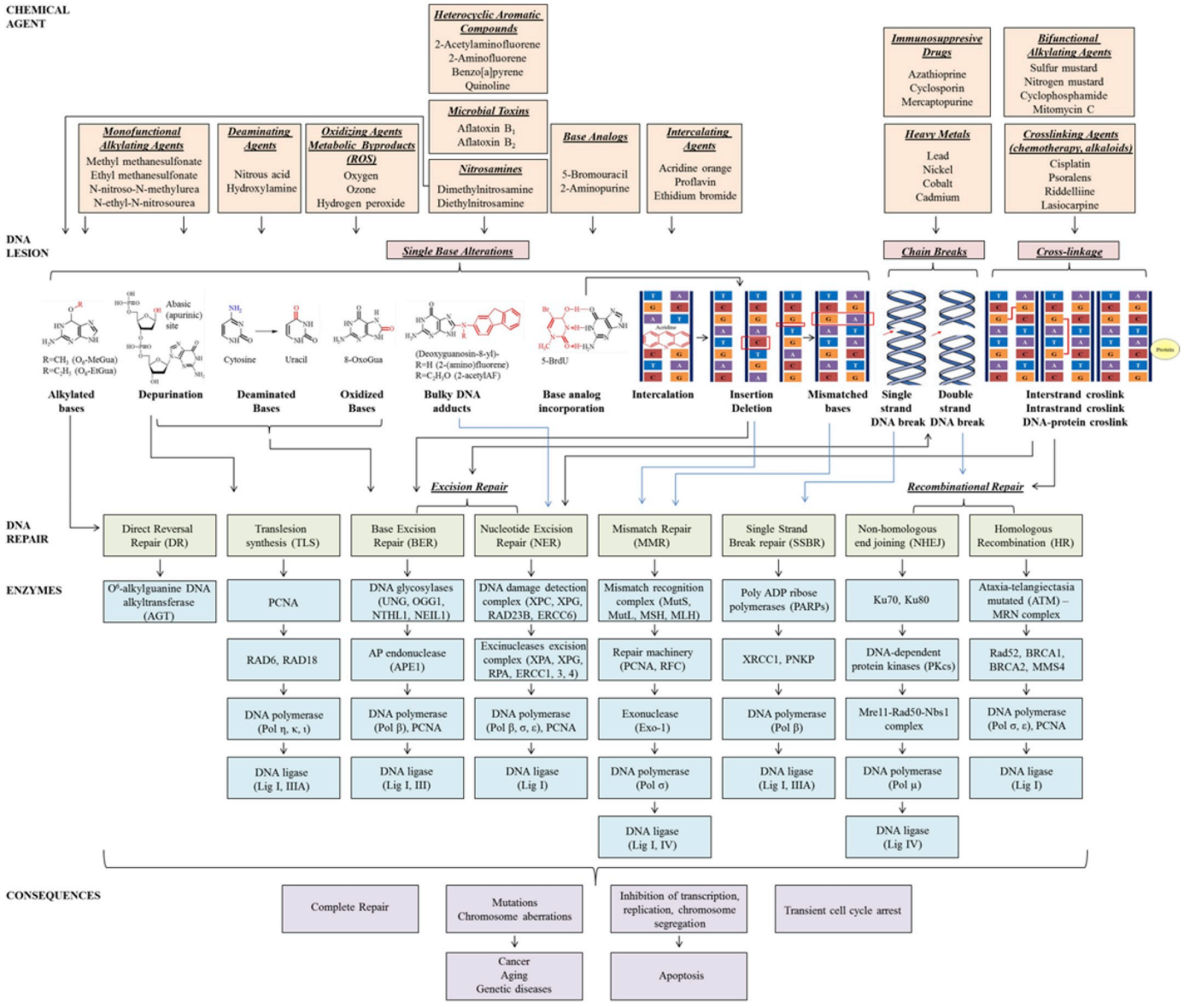

Fig. 5. DNA repair pathways for chemical induced DNA lesions.

carcinogens are known to affect cell proliferation through receptor binding [197]. The amount of carcinogen reaching a receptor is a function of chemical kinetics discussed above. An attempt made by the cell to adapt to these effects often leads to increased cell proliferation, a crucial step in tumor development [28,197,213]. Failure to achieve critical levels of receptor occupancy would constitute a threshold.

Chemical-induced genomic changes can also occur in the absence of modification in the DNA sequence (Fig. 4). For example, DNA methylation status, either global or gene specific, can be altered after exposure to various chemicals [68,199,214]. Methylation of DNA mainly occurs at CpG-islands in promoter regions of genes (DNA hypermethylation) and generally leads to the transcriptional silencing of tumor suppressor genes and other cancer related genes involved in cell cycle regulation, DNA repair, apoptosis, etc. [65–67,215]. Loss of global DNA methylation (DNA hypomethylation) occurs mainly in highly methylated genome sequences, also known as repetitive elements [216,217] and is associated with activation of normally silenced pro-oncogenes and chromatin changes that in turn cause genomic instability [215].

Interaction with nucleoproteins can also lead to epigenetic changes in gene expression [68,199]. Covalent posttranslational modifications on the N-terminal tail of histones in the form of acetylation, methylation, phosphorylation ubiquitination, or sumoylation are well described epigenetic biomarkers [218,219]. Combinations of histone modifications compose a “histone code” which can cause modifications to chromatin structure [218], thereby, affecting the gene expression profile and DNA repair processes. Depending on the type of modification and the residues that are modified, histone modification can lead to either transcriptional activation or silencing of gene expression via two major mechanisms: changing of chromatin accessibility or regulating (either positively or negatively) the binding of effector molecules [219,220].

Chemical-induced alterations in expression of microRNAs (miRNAs) have been found to play a crucial role in gene expression and in epigenetic regulation. They are also involved in cell proliferation, differentiation and death [199,221,222]. These short non-coding sequences are complementary to a part of messenger RNAs (mRNAs), and can cause gene expression silencing either by preventing mRNA translation or by increased mRNA degradation.

All of the epigenetic processes described above can interact with one another and are equally important in a multistage development of cancer [223]. For example, hypermethylation of promoter region and histone acetylation suppressed expression of miRNA-127 [224]. Conversely, certain miRNAs regulate expression of enzymes that are necessary for DNA methylation of modifications of histones [225].

The likelihood of producing these epigenetic events at low doses, however, is essentially non-existent due to the abundance of these macromolecules and their constant repletion. This is likely the basis for the often high thresholds for epigenetic carcinogens (Table 1).

In summary, there are many elements in critical cells, which limit the interaction of carcinogens with genomic targets relevant to carcinogenesis.

6.5. Genome damage repair

Genomic DNA damage in eukaryotic cells is subject to repair [226–228]. A panoply of DNA repair systems exist in eukaryotic cells, notably base excision repair, nucleotide excision repair, O^6-alkylguanine DNA alkyltransferase repair, double strand break repair and DNA mismatch repair, which correct radiation or chemical damage [229–231] (Fig. 5). Some chemical adducts are repaired more efficiently than others. Thus, ethylation at the O^6-position of guanine, which is a highly pro-mutagenic adduct, is removed by the alkyl guanine alkyl transferase process more efficiently than methylation [232]. This can lead to differences in the dose-effect for mutagenicity and carcinogenicity of different alkylating agents (see below). Repair processes are highly efficient and error free, thereby, protecting repair-competent cells from mutagenicity [233,234] and carcinogenicity [235]. Following DNA damage, excision repair starts rapidly. For example, radiation-induced repair is robust in cultured human HeLa cells at 0.5–3 h [236,237]. In primary cultures of rodent and human hepatocytes, several hundred activation-dependent DNA-reactive carcinogens, likewise, have been found to elicit substantial repair synthesis within 3 h of dosing [174,238–240] and a variety of carcinogens elicited DNA repair in cultured HeLa cells after 2.5 h dosing [241]. Repair has been shown to be more active in transcriptionally active genes than in non-coding regions [242,243], thereby preferentially protecting vulnerable sites in the genome. Consequently, DNA repair clearly plays a critical role in mitigating the level of genotoxicity of DNA-reactive carcinogens and, thereby, contributing to thresholds.

For epigenetic agents, the genomic targets are nuclear proteins, DNA (via change in methylation status) and miRNAs (see above). Importantly, carcinogens can impact methylation status of DNA repair genes leading to their epigenetic inactivation [244].

Major epigenetic alterations, DNA methylation and histone modifications, are regulated by various enzymes (Fig. 4) and thus are conceptually reversible. Some enzymes serve as “writers” inducing certain modification, while others act as “erasers” removing them. For example, DNA methyltransferases (DNM1, 3a and 3b) facilitate DNA methylation process, while ten-eleven translocation enzymes, as well as DNA glycosylases, take part in the active demethylation process [215]. Epigenetic writers, such as histone acetyltransferases, histone methyltransferases, protein arginine methyltransferases and kinases are responsible for adding epigenetic marks on histone tails. In contrast, enzymes such as histone deacetylases, lysine demethylases and protein phosphatases, catalyze the reversal of epigenetic modifications [245]. Chemical carcinogens at high doses can cause dysregulation of epigenetic enzymes, thereby leading to altered epigenetic modifications [223]. Such effects, however, have not been described at low exposures. Moreover, epigenetic modifications produced by a chemical carcinogen not only depend on the dose but can also be time-dependent, as shown with a rodent non-genotoxic carcinogen, furan [246].

6.6. Mutation and permanent alterations in gene expression

Cancer is known to involve mutation or changes in expression of a

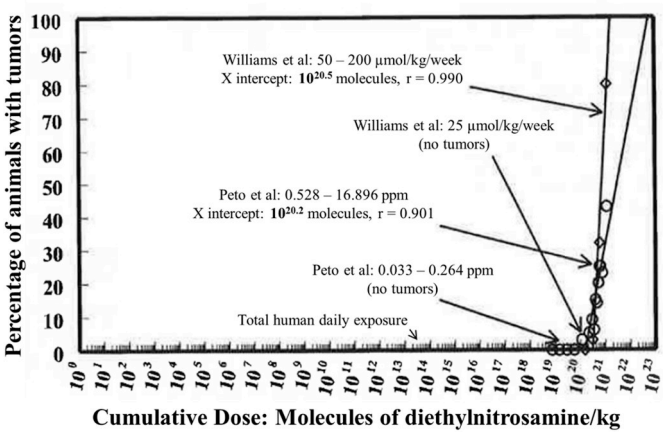

Fig. 6. Liver cell tumors in rats dosed with diethylnitrosamine (DENA). Modified from Waddell et al. [268]. Data for liver tumors from Peto et al. and Williams et al. [88,182] were plotted on the Rozman et al. [15] scale. Dose is calculated on the basis of cumulative dose in molecules/kg. The approximate average of the thresholds for DENA-induced liver tumors is $10^{20.3}$. Also shown, for perspective, is the total human daily dose of DENA from all sources estimated by Bartsch and Montesano; and Spiegelhalder and Preussmann [269,270]. The human doses are from single exposures and not cumulative doses.

variety of critical genes, suggested to be up to 8 to 10 [42,247]. These have been designated as proto-oncogenes and tumor suppressing genes [248,249]. The substantial number of gene changes required for neoplastic conversion, makes it highly unlikely that low exposures of critical cells to a chemical carcinogen would be capable of producing such numerous mutations in a single cell.

A "hit" by a chemical carcinogen in a critical gene will be converted to a permanent mutation only if DNA replication takes place before repair of the lesion. Thus, a substantial number of "hits" over an extended period of time is needed to produce the critical mutations in cells which enable them to evolve into pre-neoplastic and ultimately neoplastic populations. Such transformation occurs only if the initial pre-neoplastic cell is not removed by other protective processes (e.g., apoptosis, immunosurveilance) which provide additional barriers to cancer, as discussed below.

In cell culture studies of mutagenicity over a range of doses, the activation-independent methylating agents, methyl methanesulfonate and methylnitrosourea, dosed once showed significantly lower effects at low doses compared with expected mutant incidences estimated from a dose 5 times higher [250]. In contrast, ethylating agents yielded a linear dose-effect, which was suggested to be due to lack of efficient DNA repair of the latter. These observations of non-linearity have been elaborated in subsequent mutagenicity studies of alkylating agents in cell culture, (reviewed by Thomas and Johnson [251]). The *in vivo* mutagenicity of $MeIQ_x$ in the Big Blue transgenic rat in a target organ was shown to exhibit NOEL at the *LacI* locus of the transgene [179].

The production of mutations by chemical-specific DNA lesions discussed above, is a key effect of DNA-reactive carcinogens [90]. Mutations leading to neoplastic conversion are relevant to cancer only when they occur in cells that are the permanent residents of a tissue, namely adult stem cells [252–254].

Epigenetic carcinogens, as noted above, lack the property of direct mutagenicity, and rather act through other mechanisms [30,68,255]. The epigenetic mechanisms include enhancement of cell proliferation which leads to increases in mutation rates or facilitation of growth of background preneoplastic or neoplastic cells [256]. Permanent alterations in gene expression can arise from epigenetic effects on nucleoproteins [246]. Because of the abundance of such proteins, numerous interactions over an extended duration are required to elicit an effect, which is not consistent with the "one hit" LNT theory. Unlike some DNA-reactive carcinogens (discussed above), epigenetic carcinogens are not active with a single dose, except for highly lipophilic chemicals for which administration of a single large dose can result in a prolonged internal dose.

Thus, for both, DNA-reactive and epigenetic carcinogens a variety of factors limit induction of gene change, and this can lead to NOELs for tumor induction.

6.7. Formation of preneoplastic lesions

Preneoplastic cells and the lesions formed by them typically precede neoplasia and are usually more numerous than the neoplasms which eventually arise [257]. Various factors influence the survival and progression of such altered cells, both positively (cancer facilitators) and negatively (cancer inhibitors). Importantly, cell-to-cell communication via gap junction intercellular communication plays a major role in regulation of proliferation of normal and neoplastic cells [258]. Inhibition of this cancer protective process can be produced by tumor promoters, as first described by Yotti et al. and Murray and Fitzgerald [259,260] and confirmed by others [256,261,262], thereby facilitating growth of neoplastic cells. This appears to be a likely high-level-exposure mode of action for many epigenetic carcinogens [58,263], often referred to as promoters. Gap junctions are abundant on cell membranes, and thus, inhibition of cell-cell communication requires substantial and sustained exposure of transformed cells. Otherwise, if cell to cell communication is effective, preneoplasia can be controlled by tissue homeostatic factors and never progress to cancer. Indeed, after cessation of carcinogen dosing phenotypic reversion and disappearance of preneoplastic liver foci has been reported [257], even though such lesions are documented to have genomic alteration [31].

6.8. Systemic factors

In addition to the cellular and intercellular effects produced by carcinogens in critical cells, there are several systemic factors that influence neoplastic development, some enhancing, others inhibiting the process. These include perturbation of hormone production, chronic inflammation and immune suppression. These all represent toxicities and are not established to affect carcinogenesis at low dosages.

In other words, these systemic factors occur only at exposures above thresholds.

7. Summary of thresholds in the process of carcinogenesis

Each of the key events in chemical carcinogenesis has a threshold intrinsic to the mechanism involved. Hence, the apical event, tumor development, has a threshold level stemming from the multiple and redundant thresholds of underlying events. For DNA-reactive carcinogens, the tumor threshold can be quite low as a consequence of the potency of DNA reactivity. For epigenetic carcinogens, the threshold is generally quite high, reflecting the need for toxicity to effect carcinogenicity.

8. Conclusions

In the carcinogenicity dose-effect experiments reviewed herein, NOELs for neoplasia were observed for both DNA-reactive (eight out of fourteen) and epigenetic (seven out of seven) carcinogens at dosages below those at which tumors were induced. This is indicative of dose thresholds for both types of carcinogens. All epigenetic carcinogens exhibited tumor NOEL. For most DNA-reactive carcinogens, dose-effect experiments for tumors often revealed linear responses to the lowest dosages tested and some did not exhibit a NOEL (i.e., AAF, AFB, BaP, DMAS, ENU, NM), whereas experiments with others (DENA, DMAB, DMNA, ENUR, formaldehyde, $MeIQ_x$, RID and VC) did exhibit NOELs (Table 1). In the cases where NOELs were not observed, the lowest dosage tested was not documented to be nontoxic, i.e., not to produce genotoxic or cytotoxic effects which could induce neoplasia. Indeed, for some of these (AAF, AFB, $MeIQ_x$) mechanistic studies revealed DNA adduct formation at dosages below those tested for carcinogenicity, indicating that dosages below these toxic dosages would need to be tested to definitively assess tumor NOEL. In several studies in which thresholds were not found, the lowest dosage induced mainly or only benign tumors (e.g. benign liver tumors produced by low dosages of NM), which may reflect lesser genetic alterations, indicating that the low dosages were approaching mutagenic thresholds.

The issue of NOEL for DNA-reactive carcinogens was further addressed in studies of cancer initiation, a critical effect of DNA-reactive carcinogens. In experiments which monitored cancer initiating effects in the rat liver, almost all carcinogens, with the exceptions of VC, which was studied in a neonatal model, and dibenzo [a]pyrene in trout, demonstrated NOELs for critical effects of the DNA-reactive carcinogens studied. Importantly, in most liver initiation experiments, NOELs were found for induction of HAF, which are a prerequisite to the eventual development of liver neoplasms. Quantification of HAF provides more robust quantitative data than measurement of tumors, because, following hepatocarcinogen dosing, the numbers of HAF per liver greatly exceed the numbers of tumors [257]. Consequently, a NOEL for HAF can be considered as a NOEL for liver tumor induction, since HAF are obligatory precursors to tumors. Moreover, NOEL were demonstrated for induced cell proliferation, which is a response to hepatocellular injury and an enhancing factor in hepatocarcinogenesis [28,213].

These NOELs for induction of HAF by DNA-reactive carcinogens, as well as for increased cell proliferation, were found at CD that still produced DNA adducts, some of which are potentially miscoding, namely, (deoxyguanosin-N2-yl)-AAF with AAF [55] and O^6-ethylguanine with DEN [182], whereas others are at sites not involved in base pairing. This indicates that formation of adducts is a more sensitive bioindicator of exposure/effect and that there is a level of DNA adduct formation which appears not to lead to other toxicity. This level has been proposed to be at about 1 in 10^9 nucleotides, which represents only about three adducts per cell, or about one adduct per 7000 genes [171]. Since only 1–2% of the genome is functionally active, most adducts would be in regions of DNA not coding for gene products. Moreover, not all adducts, even in transcriptually active regions, are miscoding. Additionally, adduct levels at 1 in 10^9 nucleotides, which represents about 12 adducts per somatic cell, are extremely small compared to that of endogenous DNA lesions per cell, estimated to be in the order of 10^4 to 10^6 per cell [206,264,265], much of which is oxidative damage resulting from cellular metabolism, e.g., ROS.

Experimental chemical carcinogenesis is well established to be a multihit/multistep process (Fig. 1), involving changes in the structure or function of oncogenes and tumor suppressor genes, leading to initiation of tumor development, which can be enhanced by promotion. Such changes require, in general, substantial and sustained exposures, although there are situations where a single large dose can be tumorigenic, especially with carcinogens that are not effectively detoxicated [38,87,168]. The possibility of multiple effective hits in critical genes at extremely low levels of DNA adduct formation, as discussed above, is implausible, particularly given the effectiveness and redundancy of DNA repair processes.

Additional data that demonstrate thresholds for both DNA-reactive and epigenetic chemicals come from the analysis of altered gene expression profiles induced by exposure to carcinogens. Such analyses of genomic changes reveal NOEL for alterations of gene expression at dosages significantly lower than those already established to be NOEL for other effects, such as altered cell proliferation. In particular, the absence of induction/activation of genes related to DNA damage response, such as *Gadd*, *ATM, H2AX* [202,229], further reinforces the existence of thresholds for potentially deleterious DNA adducts [112].

Thus, the totality of the abundant biological and mechanistic evidence unequivocally supports the existence of NOELs in experimental carcinogenesis, for both tumors and antecedent effects for both DNA-reactive and epigenetic carcinogens. In contrast, there are no high quality empirical data or reliable mechanistic explanation which establish the LNT model as a biologically valid dose-effect model for all carcinogenic chemicals. Importantly, the LNT model is not consistent with the possibility of hormetic (favorable) biologic effects of chemicals at subthreshold levels for harmful effects [69,266].

9. Implications of thresholds in experimental carcinogenesis for human risk assessment

The LNT concept is frequently applied to human cancer risk assessment, where intakes generally are much lower than those in the dose-effect experiments reviewed here. This is illustrated in the dose-effect plots developed by Rozman et al. and Waddell [15,267] in which the dose is represented in molecules and a logarithmic scale is used. An example is shown in Fig. 6. Together with lower intake in humans, the factors that determine thresholds in the steps prerequisite to cancer in experimental carcinogenesis, described above, are operative, and these would be expected to afford even greater protection of humans at the much lower internal exposures resulting from lower intakes. Among these protective processes, DNA repair in cells of humans without medical conditions is more efficient than that in rodents [271,272]. The functioning of the protective processes discussed may well contribute to the fact that humans are exposed to low levels of a plethora of experimental carcinogens, both DNA reactive and epigenetic, without evidence of increased risk of cancer. Among the chemicals reviewed here, only the DNA-reactive agents, aflatoxin, BaP, formaldehyde and VC, were associated with increases in human cancer under circumstances of high intake [56,75,104,125]. Thus, the data available in humans is consistent with the existence of thresholds, and the possibility of thresholds should be considered in human risk assessment.

Declaration of interest

The authors report no conflict of interest.

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References

[1] I. Berenblum, Carcinogenesis as a Biological Problem, North Holland Publishing Co., Amsterdam, 1974.
[2] E. Farber, Sequential events in chemical carcinogenesis, in: F.F. Becker (Ed.), Cancer. A Comprehensive Treatise Etiology: Chemical and Physical Carcinogenesis, second ed., Plenum Press, NY, 1982, pp. 485–506.
[3] L. Foulds, Neoplastic Development, Academic Press, London, UK, 1969.
[4] G.M. Williams, M.J. Iatropoulos, A.M. Jeffrey, Mechanistic basis for nonlinearities and thresholds in rat liver carcinogenesis by the DNA-reactive carcinogens 2-acetylaminofluorene and diethylnitrosamine, Toxicol. Pathol. 28 (2000) 388–395.
[5] C.C. Brown, Mathematical aspects of dose-response studies in carcinogenesis—the concept of thresholds, Oncology 33 (1976) 62–65.
[6] F.W. Carlborg, The threshold and the virtually safe dose, Food ChemToxicol 20 (1982) 219–221.
[7] K.S. Crump, D.G. Hoel, C.H. Langley, R. Peto, Fundamental carcinogenic processes and their implications for low dose risk assessment, Cancer Res. 36 (1976) 2973–2979.
[8] H. Druckrey, Quantitative aspects in chemical carcinogenesis, in: R. Truhaut (Ed.), UICC Monograph Series Volume 7: Potential Carcinogenic Hazards from Drugs, Springer-Verlag Berlin, 1967.
[9] J.G. Hengstler, M.S. Bogdanffy, H.M. Bolt, F. Oesch, Challenging Dogma: thresholds for genotoxic carcinogens? The case of vinyl acetate, Annu. Rev. Pharmacol. Toxicol. 43 (2003) 495–520.
[10] W.K. Lutz, A. Kopp-Schneider, Threshold dose response for tumor induction by genotoxic carcinogens modeled via cell-cycle delay, Toxicol. Sci. 49 (1999) 110–115.
[11] N. Mantel, The concept of threshold in carcinogenesis, Clin. Pharmacol. Ther. 4 (1963) 104–109.
[12] H.G. Neumann, Risk assessment of chemical carcinogens and thresholds, Crit. Rev. Toxicol. 39 (2009) 449–461.
[13] R. Peto, R. Gray, P. Brantom, P. Grasso, Dose and time relationships for tumor induction in the liver and esophagus of 4080 inbred rats by chronic ingestion of N-nitrosodiethylamine or N-nitrosodimethylamine, Cancer Res. 51 (1991) 6452–6469.
[14] R. Preussmann, The problem of thresholds in chemical carcinogenesis; some views on theoretical and practical aspects, J. Canc. Res. Clin. Oncol. 97 (1980) 1–14.
[15] K.K. Rozman, L. Kerecsen, M.K. Viluksela, D. Österle, E. Deml, M. Viluksela, B.U. Stahl, H. Greim, J. Doull, A toxicologist's view of cancer risk assessment, Drug Metab. Rev. 28 (1996) 29–52.
[16] W. Slob, Thresholds in toxicology and risk assessment, Int. J. Toxicol. 18 (1999) 259–268.
[17] R. Truhaut, An overview of the problem of thresholds for chemical carcinogens, IARC Sci. Publ. 25 (1979) 191–202.
[18] J.H. Weisburger, G.M. Williams, Types and amounts of carcinogens as potential human cancer hazards, Cell Biol. Toxicol. 5 (1989) 377–391.
[19] G.M. Williams, Mechanistic considerations in cancer risk assessment, Inhal. Toxicol. 11 (1999) 549–554.
[20] National Academy of Science (NAS), Committee on Safe Drinking Water. Drinking Water and Health, National Academy Press, Washington, DC, 1977.
[21] E.J. Calabrese, Flaws in the LNT single-hit model for cancer risk: an historical assessment, Environ. Res. 158 (2017) 773–788.
[22] G.M. Williams, Genotoxic and epigenetic carcinogens: their identification and significance, Ann. N. Y. Acad. Sci. 407 (1983) 328–333.
[23] G.M. Williams, Mechanisms of chemical carcinogenesis and application to human cancer risk assessment, Toxicology 166 (2001) 3–10.
[24] H. Druckrey, Chemical carcinogenesis on n-nitroso derivatives, Gann Monogr. Cancer Res. 17 (1975) 107–132.

[25] P.N. Magee, E. Farber, Toxic liver injury and carcinogenesis. Methylation of rat-liver nucleic acids by dimethylnitrosamine in vivo, Biochem. J. 83 (1962) 114–124.
[26] L. Ehrenberg, P. Brookes, H. Druckrey, B. Lagerlof, J. Litwin, G. Williams, The relation of cancer induction and genetic damage, in: C. Ramel (Ed.), Evaluation of Genetic Risks of Environmental Chemicals, University of Stockholm, Stockholm, Ambio Special Report No. 3, 1973, pp. 15–16.
[27] B.E. Butterworth, T.J. Slaga, Banbury report 25: nongenotoxic mechanisms in carcinogenesis, Genet. Res. 52 (1987) 76.
[28] S.M. Cohen, L.B. Ellwein, Genetic errors, cell proliferation, and carcinogenesis, Cancer Res. 51 (1991) 6493–6505.
[29] P. Grasso, M. Sharrat, A.J. Cohen, Role of persistent, non-genotoxic tissue damage in rodent cancer and relevance to humans, Pharmacol. Toxicol. 31 (1991) 253–287.
[30] G.M. Williams, DNA reactive and epigenetic carcinogens, Exp. Toxicol. Pathol. 44 (1992) 457–464.
[31] T. Kobets, G.M. Williams, Chemicals with carcinogenic activity primarily in rodent liver, in: C.A. McQueen (Ed.), Comprehensive Toxicology, third ed., vol. 2, Elsevier Inc, Oxford, 2018, pp. 409–442.
[32] P.N. Magee, In vivo reactions of nitroso compounds, Ann NY Acad Sci 163 (1969) 717–729.
[33] J.A. Miller, Carcinogenesis by chemicals: an overview–G. H. A. Clowes memorial lecture, Cancer Res. 30 (1970) 559–576.
[34] H.G. Neumann, Ultimate electrophilic carcinogens and cellular nucleophilic reactants. A contribution to the discussion on threshold doses of environmental chemicals, Arch. Toxicol. 32 (1974) 27–38.
[35] H. Marquardt, Cell cycle dependence of chemically induced malignant transformation in vitro, Cancer Res. 34 (7) (1974) 1612–1615.
[36] J.J. Berman, C. Tong, G.M. Williams, Enhancement of mutagenesis during cell replication of cultured liver epithelial cells, Cancer Lett. 4 (5) (1978) 277–283.
[37] C. Tong, M. Fazio, G.M. Williams, Cell cycle specific mutagenesis at the hypoxanthine phosphoribosyl-transferase locus in adult rat liver epithelial cells, Proc. Natl. Acad. Sci. U. S. A. 77 (1980) 7377–7379.
[38] T. Tanaka, H. Mori, N. Hirota, K. Furuya, G.M. Williams, Effect of DNA synthesis on induction of preneoplastic and neoplastic lesions in rat liver by a single dose of methoxyazoxymethanol acetate, Chem. Biol. Interact. 58 (1986) 13–27.
[39] S.D. Vesselinovitch, K.V.N. Rao, N. Mihailovitch, Neoplastic response of mouse tissues during perinatal age periods and its significance in chemical carcinogenesis in perinatal carcinogenesis, NCI Monograph 51 (1979) 239–249.
[40] G.P. Warwick, Effect of the cell cycle on carcinogenesis, Fed. Proc. 30 (1971) 1760–1765.
[41] A. Balmain, C.C. Harris, Carcinogenesis in mouse and human cells: parallels and paradoxes, Carcinogenesis 21 (2000) 371–377.
[42] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646–674.
[43] M. Barbacid, Ras oncogenes: their role in neoplasia, Eur. J. Clin. Invest. 20 (1990) 225–235.
[44] A. Puisieux, S. Lim, J. Groopman, M. Ozturk, Selective targeting of p53 gene mutational hotspots in human cancers by etiologically defined carcinogens, Cancer Res. 51 (22) (1991) 6185–6189.
[45] G.M. Williams, M.J. Iatropoulos, C.X. Wang, A.M. Jeffrey, S. Thompson, B. Pittman, M. Palasch, R. Gebhardt, Non-linearities in 2-acetylaminofluorene exposure-responses for genotoxic and epigenetic effects leading to initiation of carcinogenesis in rat liver, Toxicol. Sci. 45 (1998) 152–161.
[46] S.D. Cline, Mitochondrial DNA damage and its consequences for mitochondrial gene expression, Biochim. Biophys. Acta 1819 (2012) 979–991.
[47] R.H. Elbekai, H.M. Korashy, K. Wills, N. Gharavi, A.O. El-Kadi, Benzo[a]pyrene, 3-methylcholanthrene and beta-naphthoflavone induce oxidative stress in hepatoma hepa 1c1c7 Cells by an AHR-dependent pathway, Free Radic. Res. 38 (11) (2004) 1191–1200.
[48] M.E. Kendall, D.P. Rose, The effects of diethylstilbestrol, tamoxifen, and toremifene on estrogen-inducible hepatic proteins and estrogen receptor proteins in female rats, Toxicol. Appl. Pharmacol. 114 (1992) 127–131.
[49] M. Kirsch-Volders, L. Gonzalez, P. Carmichael, D. Kirkland, Risk assessment of genotoxic mutagens with thresholds: a brief introduction, Mutat. Res. 678 (2009) 72–75.
[50] F. Oesch, M.E. Herrero, J.G. Hengstler, M. Lohmann, M. Arand, Metabolic detoxification: implications for thresholds, Toxicol. Pathol. 28 (2000) 382–387.
[51] S. Fukushima, H. Wanibuchi, K. Morimura, M. Wei, D. Nakae, Y. Konishi, H. Tsuda, N. Uehara, K. Imaida, T. Shirai, M. Tatematsu, T. Tsukamoto, M. Hirose, F. Furukawa, K. Wakabayashi, Y. Totsuka, Lack of a dose-response relationship for carcinogenicity in the rat liver with low doses of 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline or N-nitrosodiethylamine, Jpn. J. Canc. Res. 93 (2002) 1076–1082.
[52] G.M. Williams, Application of mode-of-action considerations in human cancer risk assessment, Toxicol. Lett. 180 (2008) 75–80.
[53] W. Lijinsky, R.M. Kovatch, C.W. Riggs, P.T. Walters, Dose-response study with N-nitrosomorpholine in drinking water of F-344 rats, Cancer Res. 48 (1988) 2089–2095.
[54] G.N. Wogan, S. Paglialunga, P.M. Newberne, Carcinogenic effects of low dietary levels of Aflatoxin B_1 in rats, Food Chem. Toxicol. 12 (1974) 681–685.
[55] G.M. Williams, J.-D. Duan, M.J. Iatropoulos, T. Kobets, A no observed adverse effect level for DNA adduct formation in rat liver with repeat prolonged dosing of the hepatocarcinogen 2-acetylaminofluorene, Toxicol. Res. 4 (2015) 233–240.
[56] International Agency for Research on cancer (IARC), IARC monographs on the evaluation of carcinogenic risks to humans. Vol 100 (A-F), Review of Human Carcinogens. Lyon, France, 2012.
[57] G.M. Williams, M.J. Iatropoulos, H.G. Enzmann, U. Deschl, Principles of testing for carcinogenic activity, in: A. Wallace Hayes, Claire L. Kruger (Eds.), Hayes' Principles and Methods of Toxicology, sixth ed., Taylor and Francis, Philadelphia, PA, 2014, pp. 1251–1303.
[58] J.E. Trosko, The role of stem cells and gap junctional intercellular communication in carcinogenesis, J. Biochem. Mol. Biol. 36 (2003) 43–48.
[59] H. Kasai, Analysis of a form of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis, Mutat. Res. 387 (1997) 147–163.
[60] J.E. Klaunig, L.M. Kamendulis, The role of oxidative stress in carcinogenesis, Annu. Rev. Pharmacol. Toxicol. 44 (2004) 239-2367.
[61] G.M. Williams, A.M. Jeffrey, Oxidative DNA damage: endogenous and chemically induced, Regul. Toxicol. Pharmacol. 32 (2000) 283–292.
[62] J.C. Corton, M.L. Cunningham, B.T. Hummer, C. Lau, B. Meek, J.M. Peters, J.A. Popp, L. Rhomberg, J. Seed, J.E. Klaunig, Mode of action framework analysis for receptor-mediated toxicity: the peroxisome proliferator-activated receptor alpha (PPARalpha) as a case study, Crit. Rev. Toxicol. 44 (2014) 1–49.
[63] H. Kasai, Y. Okada, S. Nishimura, M.S. Rao, J.K. Reddy, Formation of 8-hydroxydeoxyguanosine in liver DNA of rats following long-term exposure to a peroxisome proliferator, Cancer Res. 49 (1989) 2603–2605.
[64] M. Schrader, H.D. Fahimi, Peroxisomes and oxidative stress, Biochim. Biophys. Acta 1763 (2006) 1755–1766.
[65] M. Esteller, Epigenetic lesions causing genetic lesions in human cancer: promoter hypermethylation of DNA repair genes, Eur. J. Canc. 36 (2000) 2294–2300.
[66] M. Esteller, Epigenetic gene silencing in cancer: the DNA hypermethylome, Hum. Mol. Genet. 1 (2007) R50–R59.
[67] P.A. Jones, DNA methylation errors and cancer, Cancer Res. 56 (1996) 2463–2467.
[68] I.P. Pogribny, I. Rusyn, Environmental toxicants, epigenetics, and cancer, Adv. Exp. Med. Biol. 754 (2013) 215–232.
[69] S. Fukushima, A. Kinoshita, R. Puatanachokchai, M. Kushida, H. Wanibuchi, K. Morimura, Hormesis and dose-response-mediated mechanisms in carcinogenesis: evidence for a threshold in carcinogenicity of non-genotoxic carcinogens, Carcinogenesis 26 (2005) 1835–1845.
[70] International Expert Panel on Carcinogen Risk Assessment, The use of mechanistic data in the risk assessments of ten chemicals: an introduction to the chemical-specific reviews, Pharmacol. Ther. 71 (1996) 1–5.
[71] K.T. Kitchin, J.L. Brown, R.W. Setzer, Dose-response relationship in multistage carcinogenesis: promoters, Environ. Health Perspect. Suppl. 102 (1994) 255–264.
[72] J. Whysner, G.M. Williams, Butylated hydroxyanisole mechanistic data and risk assessment: conditional species-specific cytotoxicity, enhanced cell proliferation, and tumor promotion, Pharmacol. Ther. 71 (1996) 137–151.
[73] J.A. Swenberg, Alpha 2u-globulin nephropathy: review of the cellular and molecular mechanisms involved and their implications for human risk assessment, Environ. Health Perspect. 101 (Suppl 6) (1993) 39–44.
[74] C.E. Perrone, L. Shao, G.M. Williams, Effect of rodent hepatocarcinogenic peroxisome proliferators on fatty Acyl-CoA oxidase, DNA synthesis and apoptosis in cultured human and rat hepatocytes, Toxicol. Appl. Pharmacol. 150 (1998) 277–286.
[75] International Agency for Research on cancer (IARC), Vinyl chloride, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol 100F, Chemical Agents and Related Occupations. Lyon, France, 2012, pp. 451–478.
[76] P. Bannasch, Preneoplastic lesions as end points in carcinogenicity testing. I. Hepatic preneoplasia, Carcinogenesis 7 (1986) 689–695.
[77] F.C. Richardson, P.H. Morgan, J.A. Boucheron, F.H. Deal, J.A. Swenberg, Hepatocyte initiation during continuous administration of diethylnitrosamine and 1,2-*syn*-dimethylhydrazine, Cancer Res. 48 (1988) 988–992.
[78] G.M. Williams, Phenotypic properties of preneoplastic rat liver lesions and applications to detection of carcinogens and tumor promoters, Toxicol. Pathol. 10 (1982) 3–10.
[79] G.M. Williams, M.J. Iatropoulos, C.X. Wang, N. Ali, A. Rivenson, L.A. Peterson, C. Schulz, R. Gebhardt, Diethylnitrosamine exposure-responses for DNA damage, centrilobular cytotoxicity, cell proliferation and carcinogenesis in rat liver exhibit some non-linearities, Carcinogenesis 17 (1996) 2253–2258.
[80] P. Greaves, R. Goonetilleke, G. Nunn, J. Topham, T. Orton, Two-year carcinogenicity study of tamoxifen in Alderley Park Wistar-derived rats, Cancer Res. 53 (17) (1993) 3919–3924.
[81] F. Montandon, G.M. Williams, Comparison of DNA reactivity of the polyphenylethylene hormonal agents diethylstilbestrol, tamoxifen and toremifene in rat and hamster liver, Arch. Toxicol. 68 (1994) 272–275.
[82] International Agency for Research on Cancer (IARC), *N*-Nitrosodiethylamine, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some N-nitroso Compounds, vol. 17, 1978, pp. 83–124 Lyon, France.
[83] L. Verna, J. Whysner, G.M. Williams, N-Nitrosodiethylamine mechanistic data and risk assessment: bioactivation, DNA-adduct formation, mutagenicity, and tumor initiation, Pharmacol. Ther. 71 (1996) 57–82.
[84] H. Druckrey, Quantitative grundlagen der krebserzeugung, Klin Wschr 22 (1943) 532.
[85] H. Druckrey, K. Küpfmüller, Quantitative analyse der Krebsentstehung, Z. Naturforsch. 3b (1948) 254–266.
[86] H. Druckrey, D. Schmähl, W. Dischler, Dosis-Wirkungsbeziehungen b ei der Krebscrzeugung durch 4-Dimethylaminostilben bei Ratten, Z. Krebsforsch. 65 (1963) 272.
[87] H. Druckrey, D. Steinhoff, R. Preussmann, S. Ivankovic, Induction of cancer by a single dose of methylnitrosourea and various dialkylnitrosamines in rats, Z.

Krebsforsch. 66 (1964) 1–10.
[88] R. Peto, R. Gray, P. Brantom, P. Grasso, Effects on 4080 rats of chronic ingestion of N-nitrosodiethylamine or N-nitrosodimethylamine: a detailed dose-response study, Cancer Res. 51 (1991) 6415–6451.
[89] International Agency for Research on Cancer (IARC), Aflatoxins, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene, vol. 82, 2002, pp. 171–300 Lyon, France.
[90] R.J. Preston, G.M. Williams, DNA-reactive carcinogens: mode of action and human cancer hazard, Crit. Rev. Toxicol. 35 (2005) 673–683.
[91] International Agency for Research on Cancer (IARC), Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, vol. 56, 1993, pp. 1–599 Lyon, France.
[92] Z.A. Wong, D.P. Hsieh, The comparative metabolism and toxicokinetics of aflatoxin B1 in the monkey, rat, and mouse, Toxicol. Appl. Pharmacol. 55 (1980) 115–125.
[93] P. Buss, M. Caviezel, W.K. Lutz, Linear dose-response relationship for DNA adducts in rat liver from chronic exposure to aflatoxin B1, Carcinogenesis 11 (1990) 2133–2135.
[94] National Toxicology Program (NTP), Report on Carcinogens, Thirteenth Edition. NTP, Research Triangle Park, NC, USA, Department of Health and Human Services, Public Health Service, 2014 Published online http://ntp.niehs.nih.gov/pubhealth/roc/roc13/.
[95] T. Cairns, The Ed_{01} Study: introduction, objectives and experimental design, J. Environ. Pathol. Toxicol. 3 (1980) 1–7.
[96] J.H. Farmer, R.L. Kodell, D.L. Greenman, G.W. Shaw, Dose and time responses models for the incidence of bladder and liver neoplasms in mice fed 2-acetylaminofluorene continuously, J. Environ. Pathol. Toxicol. 3 (1979) 55–68.
[97] R.L. Kodell, J.H. Farmer, N.A. Littlefield, Analysis of life-shortening effects in female BALB/c mice fed 2-acetylaminofluorene, in: J.A. Staffa, M.A. Mehlman (Eds.), Innovations in Cancer Risk Assessment (ED_{01} Study), Pathotox Publishers, Park Forest South, IL, 1979, pp. 69–87.
[98] C.H. Frith, A.D. Boothe, Correaltions between gross and microscopic lesions in carcinogenic studies in mice, in: J.A. Staffa, M.A. Mehlman (Eds.), Innovations in Cancer Risk Assessment (ED_{01} Study), Pathotox Publishers, Park Forest South, IL, 1979, pp. 139–153.
[99] N.A. Littlefield, J.H. Farmer, D.W. Gaylor, W.G. Sheldon, Effects of dose and time in a long-term, low-dose carcinogenic study, J. Environ. Pathol. Toxicol. 3 (3 Spec No) (1980) 17–34.
[100] M.C. Poirier, N.F. Fullerton, T. Kinouchi, B.A. Smith, F.A. Beland, Comparison between DNA adduct formation and tumorigenesis in livers and bladders of mice chronically fed 2-acetylaminofluorene, Carcinogenesis 12 (1991) 895–900.
[101] J. Whysner, C.C. Conaway, L.K. Verna, G.M. Williams, Vinyl chloride mechanistic data and risk assessment: DNA reactivity and cross-species quantitative risk extrapolation, Pharmacol. Ther. 71 (1996) 7–28.
[102] International Agency for Research on Cancer (IARC), Senecio species and riddelliine, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene, vol. 82, 2002, pp. 153–168 Lyon, France.
[103] C. Maltoni, G. Lefemine, A. Ciliberti, G. Cotti, D. Carretti, Carcinogenicity bioassays of vinyl chloride monomer: a model of risk assessment on an experimental basis, Environ. Health Perspect. 41 (1981) 3–29.
[104] International Agency for Research on Cancer (IARC), Formaldehyde, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol, vol. 88, 2006, pp. 39–325 Lyon, France.
[105] H.D. Heck, E.L. White, M. Casanova-Schmitz, Determination of formaldehyde in biological tissues by gas chromatography/mass spectrometry, Biomed. Mass Spectrom. 9 (1982) 347–353.
[106] C.C. Conaway, J. Whysner, G.M. Williams, Formaldehyde mechanistic data and risk assessment: endogenous protection from DNA adduct formation, Pharmacol. Ther. 71 (1996) 29–56.
[107] R. Golden, Identifying an indoor air exposure limit for formaldehyde considering both irritation and cancer hazards, Crit. Rev. Toxicol. 41 (2011) 672–721.
[108] W.D. Kerns, K.L. Pavkov, D.J. Donofrio, M.M. Connell, R.I. Mitchell, E.J. Gralia, J.A. Swenberg, Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure, Cancer Res. 43 (1983) 4382–4392.
[109] T.M. Monticello, J.A. Swenberg, E.A. Gross, J.R. Leininger, J.S. Kimbell, S. Seilkop, T.B. Starr, J.E. Gibson, K.T. Morgan, Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells, Cancer Res. 56 (5) (1996) 1012–1022.
[110] E. Kamata, M. Nakadate, O. Uchida, Y. Ogawa, S. Suzuki, T. Kaneko, M. Saito, Y. Kurokawa, Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fisher-344 rats, J. Toxicol. Sci. 22 (1997) 239–254.
[111] M.E. Andersen, H.J. Clewell III, E. Bermudez, G.A. Wilson, R.S. Thomas, Genomic signatures and dose-dependent transitions in nasal epithelial responses to inhaled formaldehyde in the rat, Toxicol. Sci. 105 (2008) 368–383.
[112] R.A. Clewell, M.E. Andersen, Approaches for characterizing threshold dose-response relationships for DNA-damage pathways involved in carcinogenicity in vivo and micronuclei formation in vitro, Mutagenesis 31 (2016) 333–340.
[113] International Agency for Research on Cancer (IARC), *N*-Nitroso-*N*-ethylurea, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some N-nitroso Compounds, vol. 17, 1978, pp. 191–216 Lyon, France.
[114] A. Maekawa, T. Ogiu, C. Matsuoka, H. Onodera, K. Furuta, Y. Kurokawa, M. Takahashi, T. Kokubo, H. Tanigawa, Y. Hayashi, Carcinogenicity of low doses of N-ethyl-N-nitrosourea in F344 rats: a dose-response study, Gann 75 (1984) 117–125.
[115] International Agency for Research on Cancer (IARC), *N'*-Nitrosomorpholine, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some N-nitroso Compounds, vol. 17, 1978, pp. 263–280 Lyon, France.
[116] W. Lijinsky, Chemistry and Biology of N-nitroso Compounds, Cambridge Univ Press, NY, USA, 1992.
[117] A. Maekawa, H. Onodera, Y. Matsushima, T. Nagaoka, A. Todate, M. Shibutani, Y. Kodama, Y. Hayashi, Dose-response carcinogenicity in rats on low-dose levels of N-ethyl-N-nitrosourethane, Jpn. J. Canc. Res. 80 (1989) 632–636.
[118] International Agency for Research on Cancer (IARC), *N*-Nitrosodimethylamine, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some N-nitroso Compounds, vol. 17, 1978, pp. 125–175 Lyon, France.
[119] M.W. Chou, J. Yan, J. Nichols, Q. Xia, F.A. Beland, P.C. Chan, P.P. Fu, Correlation of DNA adduct formation and riddelliine-induced liver tumorigenesis in F344 rats and B6C3F1 mice [Cancer Lett. 193 (2003) 119-125], Cancer Lett. 207 (1) (2004) 119–125.
[120] National Toxicology Program (NTP), NTP Technical Report on the Toxicology and Carcinogenesis Studies of Riddelliine (CAS No. 23246-96-0) in F344/N Rats and B6C3F1 Mice (Gavage Studies). NTP TR508 NIH Publication No. 03-4442, National Institutes of Health, Public Health Service, US Department of Health and Human Services, 2003, pp. 1–280.
[121] International Agency for Research on Cancer (IARC), Riddeleliine, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Some Naturally Occurring Substances, vol. 10, 1976, pp. 313–317 Lyon, France.
[122] P.C. Chan, J.K. Haseman, J.D. Prejean, A. Nyska, Toxicity and carcinogenicity of riddelliine in rats and mice, Toxicol. Lett. 144 (3) (2003) 295–311.
[123] International Agency for Research on Cancer (IARC), Working Group on the Evaluation of Carcinogenic Risk to Humans, MelQx (2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Naturally Occurring Substances. Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins, vol. 56, IARC, Lyon, France, 1993, pp. 211–228.
[124] T. Murai, S. Mori, J.S. Kang, K. Morimura, H. Wanibuchi, Y. Totsuka, S. Fukushima, Evidence of a threshold-effect for 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline liver carcinogenicity in F344/DuCrj rats, Toxicol. Pathol. 36 (2008) 472–477.
[125] International Agency for Research on cancer (IARC), Benzo[a]pyrene, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol 92, Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures, 2010, pp. 394–423 Lyon, France.
[126] P.W. Wester, J.J. Muller, W. Slob, G.R. Mohn, P.M. Dortant, E.D. Kroese, Carcinogenic activity of benzo[a]pyrene in a 2 year oral study in Wistar rats, Food Chem. Toxicol. 50 (2012) 927–935.
[127] S.M. Cohen, Human relevance of animal carcinogenicity studies, Regul. Toxicol. Pharmacol. 21 (1995) 75–80.
[128] International Agency for Research on Cancer (IARC), Some chemicals that cause tumors of the kidney or urinary bladder in rodents and some other substances. Saccharin and its salts, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 73, 1999, pp. 517–624 Lyon, France.
[129] J. Whysner, G.M. Williams, Saccharin mechanistic data and risk assessment: urine composition, enhanced cell proliferation and tumor promotion, Pharmacol. Ther. 71 (1996) 225–252.
[130] J. Whysner, G.M. Williams, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin mechanistic data and risk assessment: gene regulation, cytotoxicity, enhanced cell proliferation, and tumor promotion, Pharmacol. Ther. 71 (1996) 193–224.
[131] J.M. Taylor, M.A. Weinberger, L. Friedman, Chronic toxicity and carcinogenicity to the urinary bladder of sodium saccharin in the *in utero*-exposed rat, Toxicol. Appl. Pharmacol. 54 (1980) 57–75.
[132] International Agency for Research on Cancer (IARC), Some thyrotropic agents, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 79, Amitrole, Lyon, France, 2001, pp. 381–410.
[133] D. Steinhoff, H. Weber, U. Mohr, K. Boehme, Evaluation of amitrole (aminotriazole) for potential carcinogenicity in orally dosed rats, mice, and golden hamsters, Toxicol. Appl. Pharmacol. 69 (2) (1983) 161–169.
[134] International Agency for Research on Cancer (IARC), Butylated hydroxyanisole (BHA), IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation, vol. 40, 1986, pp. 123–160 Lyon, France.
[135] G.M. Williams, C.A. McQueen, C. Tong, Toxicity studies of butylated hydroxyanisole and butylated hydroxytoluene I. Genetic toxicology studies, Fd Chem. Toxicol. 28 (1990) 793–798.
[136] N. Ito, A. Hagiwara, M. Shibata, T. Ogiso, S. Fukushima, Induction of squamous cell carcinomas in the forestomach of F344 rats treated with butylated hydroxyanisole, Gann 73 (1982) 332–334.
[137] N. Ito, S. Fukushima, S. Tamano, M. Hirose, A. Hagiwara, Dose response in butylated hydroxyanisole induction of forestomach carcinogenesis in F344 rats, J. Natl. Cancer Inst. 77 (1986) 1261–1265.
[138] J. Whysner, C. Wang, E. Zang, M.J. Iatropoulos, G.M. Williams, Dose response of promotion by butylated hydroxyanisole in chemically initiated tumors of the rat forestomach, Fd ChemToxicol 32 (1994) 215–222.
[139] International Agency for Research on Cancer (IARC), Acrylonitrile, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 71, 1999, pp. 43–108 Lyon, France. Pt 1.
[140] J. Whysner, P.M. Ross, C.C. Conaway, L.K. Verna, G.M. Williams, Evaluation of

possible genotoxic mechanisms for acrylonitrile tumorigenicity, Regul. Toxicol. Pharmacol. 27 (1998) 217–239.
[141] G.M. Williams, T. Kobets, J.D. Duan, M.J. Iatropoulos, Assessment of DNA binding and oxidative DNA damage by acrylonitrile in two rat target tissues of carcinogenicity: implications for the mechanism of action, Chem. Res. Toxicol. 30 (2017) 1470–1480.
[142] J. Whysner, R.E. Steward III, D. Chen, C.C. Conaway, L.K. Verna, J.P. Richie Jr., N. Ali, G.M. Williams, Formation of 8-oxodeoxyguanosine in brain DNA of rats exposed to acrylonitrile, Arch. Toxicol. 72 (1998) 429–438.
[143] L.M. Kamendulis, J. Jiang, Y. Xu, J.E. Klaunig, Induction of oxidative stress and oxidative damage in rat glial cells by acrylonitrile, Carcinogenesis 20 (1999) 1555–1560.
[144] F.R. Johannsen, G.J. Levinskas, Chronic toxicity and oncogenic dose-response effects of lifetime oral acrylonitrile exposure to F344 rats, Toxicol. Lett. 13 (2002) 221–247.
[145] International Agency for Research on Cancer (IARC), Some industrial chemicals. Ethylbenzene, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 77, 2000, pp. 227–266 Lyon, France.
[146] L. Henderson, D. Brusick, F. Ratpan, G. Veenstra, A review of the genotoxicity of ethylbenzene, Mutat. Res. 635 (2–3) (2007) 81–89.
[147] National Toxicology Program (NTP), NTP Technical Report on the Toxicology and Carcinogenesis Studies of Ethylbenzene (CAS No. 100-41-4) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). NTP TR466 NIH Publication No. 99-3956, National Institutes of Health, Public Health Service, US Department of Health and Human Services, 1999, pp. 1–231.
[148] P.C. Chan, J.K. Haseman, J. Mahleri, C. Aranyi, Tumor induction in F344/N rats and B6C3F1 mice following inhalation exposure to ethylbenzene, Toxicol. Lett. 99 (1) (1998) 23–32.
[149] International Agency for Research on Cancer (IARC), Some chemicals that cause tumors of the kidney or urinary bladder in rodents and some other substances. Chloroform, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 73, 1999, pp. 131–182 Lyon, France.
[150] S. Yamamoto, T. Kasai, M. Matsumoto, T. Nishizawa, H. Arito, K. Nagano, T. Matsushima, Carcinogenicity and chronic toxicity in rats and mice exposed to chloroform by inhalation, J. Occup. Health 44 (2002) 283–293.
[151] R.J. Golden, S.E. Holm, D.E. Robinson, P.H. Julkunen, E.A. Reese, Chloroform mode of action: implications for cancer risk assessment, Regul. Toxicol. Pharmacol. 26 (1997) 142–155.
[152] International Agency for Research on Cancer (IARC), Polychlorinated Dibenzo-*para*-Dioxins and Polychlorinated Dibenzofurans, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 69, 1997, pp. 33–343 Lyon, France.
[153] National Research Council (NRC), Health Risks from Dioxin and Related Compounds. Evaluation of the EPA Reassessment, National Academies Press, Washington DC, 2006.
[154] National Toxicology Program (NTP), NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2,3,7,8-tetrachlorobidenzo-p-dioxin (TCDD) CAS No. 1746-01-6) in Female Harlan Sprague-Dawley Rats (Gavage Studies). NTP TR521 NIH Publication No. 06-4468 National Institutes of Health, Public Health Service, US Department of Health and Human Services, 2006, pp. 1–232.
[155] H.C. Pitot, T.L. Goldsworthy, S. Moran, W. Kennan, P.H. Glauert, R.R. Maronpot, H.A. Campbell, A method to quantitate the relative initiating and promoting potencies of hepatocarcinogenic agents in their dose-response relationships to altered hepatic foci, Carcinogenesis 8 (1987) 1491–1499.
[156] Society of Toxicology ED Task Force, Re-examination of the ED_{01} study, Fund. Appl. Toxicol. 1 (1981) 26–126.
[157] R.L. Kodell, D.W. Gaylor, D.L. Greenman, N.A. Littlefield, J.H. Farmer, Response to the society of toxicology task force re-examination of the ED01 study, Fund. Appl. Toxicol. 3 (4) (1983) 3a–12a.
[158] L. Zeise, R. Wilson, E.A. Crouch, Dose-response relationships for carcinogens: a review, Environ. Health Perspect. 73 (1987) 259–306.
[159] T. Goldsworthy, H.A. Campbell, H.C. Pitot, The natural history and dose-response characteristics of enzyme-altered foci in rat liver following phenobarbital and diethylnitrosamine administration, Carcinogenesis 5 (1984) 67–71.
[160] H. Enzmann, E. Bomhard, M.J. Iatropoulos, H.J. Ahr, G. Schlueter, G.M. Williams, Short- and intermediate-term carcinogenicity testing - a review: Part 1 - the prototypes mouse skin tumour assay and rat liver focus, Food Chem. Toxicol. 36 (1998) 979–995.
[161] H. Enzmann, M.J. Iatropoulos, K.D. Brunnemann, E. Bomhard, H.J. Ahr, G. Schlüter, G.M. Williams, Short- and intermediate-term carcinogenicity testing – a review: Part 2 – available experimental models, Food Chem. Toxicol. 36 (1998) 997–1013.
[162] G.M. Williams, Chemically induced rodent preneoplastic lesions as indicators of carcinogenic activity McGregor DB, in: J.M. Rice, S. Venitt (Eds.), The Use of Short-and Medium-term Tests for Carcinogens and Data on Genetic Effects in Carcinogenic Hazard Evaluation, IARC Scientific Publications No. 146, Lyon, France, 1999, pp. 185–202.
[163] G.M. Williams, The significance of chemically-induced hepatocellular altered foci in rat liver and application to carcinogen detection, Toxicol. Pathol. 17 (1989) 663–674.
[164] S. Fukushima, G. Min, A. Kakehashi, H. Wanibuchi, Qualitative and quantitative assessments on low-dose carcinogenicity of genotoxic hepatocarcinogens: dose-response for key events in rat hepatocarcinogenesis, in: T. Nohmi, S. Fukushima (Eds.), Thresholds of Genotoxic Carcinogens: from Mechanisms to Regulation, Elsevier, 2016.
[165] T. Kobets, G.M. Williams, Thresholds for hepatocarcinogenicity of DNA-reactive compounds, in: T. Nohmi, S. Fukushima (Eds.), Thresholds of Genotoxic Carcinogens: from Mechanisms to Regulation, Elsevier, 2016.
[166] G.M. Williams, M.J. Iatropoulos, A.M. Jeffrey, Dose-effect relationships for DNA-reactive liver carcinogens, in: H. Greim, R.J. Albertini (Eds.), The Cellular Reposnse to the Genotoxic Insult: the Question of Threshold for Genotoxic Carcinogens, Royal Society of Chemistry Issues in Toxicology Series, 2012, pp. 33–51.
[167] H. Mori, T. Tanaka, S. Sugie, M. Takashashi, G.M. Williams, DNA content of liver cell nuclei of N-2-fluorenylacetamide-induced altered foci and neoplasms in rats and human hyperplastic foci, J. Natl. Cancer Inst. 69 (1982) 1277–1282.
[168] H.C. Pitot, L. Barsness, T. Goldworthy, T. Kitagawa, Biochemical characterization of stages of hepatocarcinogenesis after a single dose of diethylnitrosamine, Nature 271 (1978) 456–458.
[169] G.M. Williams, Functional markers and growth behavior of preneoplastic hepatocytes, Cancer Res. 36 (1976) 2540–2543.
[170] H. Tsuda, S. Fukushima, H. Wanibuchi, K. Morimura, D. Nakae, K. Imaida, M. Tatematsu, M. Hirose, K. Wakabayashi, M.A. Moore, Value of GST-P positive preneoplastic hepatic foci in dose-response studies of hepatocarcinogenesis: evidence for practical thresholds with both genotoxic and nongenotoxic carcinogens. A review of recent work, Toxicol. Pathol. 31 (2003) 80–86.
[171] G.M. Williams, M.J. Iatropoulos, A.M. Jeffrey, Thresholds for DNA-reactive (genotoxic) organic carcinogens, J. Toxicol. Pathol. 18 (2005) 69–77.
[172] O. Hino, T. Kitagawa, Existence of a practical threshold dose for the hepatocarcinogen 3'-methyl-4-(dimethylamino) azobenzene in rat liver, Gann 72 (1981) 637–638.
[173] International Agency for Research on Cancer (IARC), *para*-Dimethylaminoazobenzene, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Some Aromatic Azo Compounds, vol. 8, 1975, pp. 125–146 Lyon, France.
[174] G.M. Williams, H. Mori, C.A. Mcqueen, Structure-activity relationships in the rat hepatocyte DNA-repair test for 300 chemicals, Mutat. Res. 221 (3) (1989) 263–286.
[175] G.E. Dunaif, T.C. Campbell, Relative contribution of dietary protein level and aflatoxin B_1 dose in generation of presumptive preneoplastic foci in rat liver, JNCL 78 (1987) 365–369.
[176] M. Root, T. Lange, T.C. Campbell, Dissimilarity in aflatoxin dose-responser-elationships between DNA adduct formation and development of preneoplastic foci in rat liver, Chem. Biol. Interact. 106 (1997) 213–227.
[177] H. Enzmann, H. Zerban, A. Kopp-Schneider, E. Loser, P. Bannach, Effects of low doses of N-nitrosomorpholine on the development of early stages of hepatocarcinogenesis, Carcinogenesis 16 (1995) 1513–1518.
[178] S. Fukushima, Low-dose carcinogenicity of a heterocyclic amine, 2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline: relevance to risk assessment, Cancer Lett. 143 (1999) 157–159.
[179] M. Hoshi, K. Morimura, H. Wanibuchi, M. Wei, E. Okochi, T. Ushijima, K. Takaoka, S. Fukushima, No-observed effect levels for carcinogenicity and for in vivo mutagenicity of a genotoxic carcinogen, Toxicol. Sci. 81 (2004) 273–279.
[180] R.J. Laib, T. Pellio, V.M. Wünschel, N. Zimmermann, H.M. Bolt, The rat liver foci bioassay: II. Investigations on the dose-dependent induction of ATPase-deficient foci by vinyl chloride at very low doses, Carcinogenesis 6 (1985) 69–72.
[181] G.M. Williams, R. Gebhardt, H. Sirma, F. Stenbäck, Non-linearity of neoplastic conversion induced in rat liver by low exposures to diethylnitrosamine, Carcinogenesis 14 (1993) 2149–2156.
[182] G.M. Williams, M.J. Iatropoulos, A.M. Jeffrey, F.Q. Luo, C.X. Wang, B. Pittman, Diethylnitrosamine exposure-responses for DNA ethylation, hepatocellular proliferation and initiation of carcinogenesis in rat liver display non-linearities and thresholds, Arch. Toxicol. 73 (1999) 394–402.
[183] A. Loveless, Possible relevance of O-6 alkylation of deoxyguanosine to the mutagenicity and carcinogenicity of nitrosamines and nitrosamides, Nature 223 (1969) 206–207.
[184] G.M. Williams, M.J. Iatropoulos, A.M. Jeffrey, Thresholds for the effects of 2-acetylaminofluorene in rat liver, Toxicol. Pathol. 32 (Suppl. 2) (2004) 85–91.
[185] G.S. Bailey, A.P. Reddy, C.B. Pereira, V. Harttig, W. Baird, J.M. Spitzbergen, J.D. Hendricks, G.A. Orner, D.E. Williams, J.A. Swenberg, Nonlinear cancer response of ultralow dose: a 40,800-animal ED001 tumor and biomarker study, Chem. Res. Toxicol. 22 (2009) 1264–1276.
[186] C. Masuda, H. Wanibuchi, K. Otori, M. Wei, S. Yamamoto, T. Hiroi, S. Imaoka, Y. Funae, S. Fukushima, Presence of a no-observe effect level for enhancing effect of development of the α-isomer of benzene hexachloride (α-BHC) on diethylnitrosamine-initiated hepatic foci in rats, Cancer Lett. 163 (2001) 179–185.
[187] M. Muguruma, M. Kawai, Y. Dewa, J. Nishimura, Y. Saegusa, H. Yasuno, M. Jin, S. Matsumoto, M. Takabatake, K. Arai, K. Mitsumori, Threshold dose of piperonyl butoxide that induces reactive oxygen species-mediated hepatocarcinogenesis in rats, Arch. Toxicol. 83 (2009) 183–193.
[188] R.T. Drew, G.A. Boorman, J.K. Haseman, E.E. McConnell, W.M. Busy, J.A. Moore, The effect of age and exposure duration on cancer induction by a known carcinogen in rats, mice and hamsters, Toxicol. Appl. Pharmacol. 68 (1983) 120–130.
[189] D.K. Sinha, T.L. Dao, Induction of mammary tumors in aging rats by 7,12-dimethyl-penz(a)anthracene: role of DNA synthesis during carcinogenesis, J. Natl. Cancer Inst. 64 (1980) 519–521.
[190] D.G. Walton, A.B. Acton, H.F. Stich, DNA repair synthesis in cultured fish and human cells exposed to fish S9-activated aromatic hydrocarbons, Comp. Biochem. Physiol., C 86 (1987) 399–404.
[191] E. Bianconi, A. Piovesan, F. Facchin, A. Beraudi, R. Casadei, F. Frabetti, L. Vitale, M.C. Pelleri, S. Tassani, F. Piva, S. Perez-Amodio, P. Strippoli, S. Canaider, An estimation of the number of cells in the human body, Ann. Hum. Biol. 40 (2013)

463–471.
[192] F.P. Guengerich, Cytochrome p450 and chemical toxicology, Chem. Res. Toxicol. 21 (1) (2008) 70–83.
[193] D.F. Lewis, C. Ioannides, D.V. Parke, Cytochromes P450 and species differences in xenobiotic metabolism and activation of carcinogen, Environ. Health Perspect. 106 (1998) 633–641.
[194] M.J. Iatropoulos, G.M. Williams, Proliferation markers, Exp. Toxicol. Pathol. 48 (2–3) (1996) 175–181.
[195] W.M. Wong, N.A. Wright, Cell proliferation in gastrointestinal mucosa, J. Clin. Pathol. 52 (5) (1999) 321–333.
[196] F.J. Sharom, The P-glycoprotein multidrug transporter, Essays Biochem. 50 (2011) 161–178.
[197] G.M. Williams, M.J. Iatropoulos, Alteration of liver cell function and proliferation: differentiation between adaptation and toxicity, Toxicol. Pathol. 30 (2002) 41–53.
[198] W.J. Waddell, Thermodynamic basis for expressing dose logarithmically, Toxicol. Appl. Pharmacol. 228 (2008) 156–157.
[199] G. Chappell, I.P. Pogribny, K.Z. Guyton, I. Rusyn, Epigenetic alterations induced by genotoxic occupational and environmental human chemical carcinogens: a systematic literature review, Mutat. Res. Rev. Mutat. Res. 768 (2016) 27–45.
[200] C. Biémont, A brief history of the status of transposable elements: from junk DNA to major players in evolution, Genetics 186 (2010) 1085–1093.
[201] R.E. Thurman, E. Rynes, R. Humbert, J. Vierstra, M.T. Maurano, E. Haugen, N.C. Sheffield, A.B. Stergachis, H. Wang, B. Vernot, K. Garg, S. John, R. Sandstrom, D. Bates, L. Boatman, T.K. Canfield, M. Diegel, D. Dunn, A.K. Ebersol, T. Frum, E. Giste, A.K. Johnson, E.M. Johnson, T. Kutyavin, B. Lajoie, B.K. Lee, K. Lee, D. London, D. Lotakis, S. Neph, F. Neri, E.D. Nguyen, H. Qu, A.P. Reynolds, V. Roach, A. Safi, M.E. Sanchez, A. Sanyal, A. Shafer, J.M. Simon, L. Song, S. Vong, M. Weaver, Y. Yan, Z. Zhang, Z. Zhang, B. Lenhard, M. Tewari, M.O. Dorschner, R.S. Hansen, P.A. Navas, G. Stamatoyannopoulos, V.R. Iyer, J.D. Lieb, S.R. Sunyaev, J.M. Akey, P.J. Sabo, R. Kaul, T.S. Furey, J. Dekker, G.E. Crawford, J.A. Stamatoyannopoulos, The accessible chromatin landscape of the human genome, Nature 489 (7414) (2012) 75–82.
[202] A.J. Fornace, D.W. Nebert, M.C. Hollander, J.D. Luethy, M. Papthanasiou, J. Farqnoli, N.J. Holbrook, Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents, Mol. Cell Biol. 9 (1989) 4196–4203.
[203] M.J. Hickman, L.D. Samson, Role of DNA mismatch repair and p53 in signaling induction of apoptosis by alkylating agents, Proc. Nat. Acad. Sci. USA 96 (1999) 10764–10769.
[204] B.N. Ames, Endogenous DNA damage as related to cancer and aging, Mutat. Res. 214 (1989) 41–46.
[205] T. Lindahl, A. Andersson, Rate of chain breakage at apurinic sites in double-stranded deoxyribonucleic acid, Biochemistry 11 (1972) 3618–3623.
[206] L.J. Marnett, P.C. Burcham, Endogenous DNA adducts: potential and paradox, Chem. Res. Toxicol. 6 (1993) 771–785.
[207] J.A. Swenberg, K. Lu, B.C. Moeller, L. Gao, P.B. Upton, J. Nakamura, T.B. Starr, Endogenous versus exogenous DNA adducts: their role in carcinogenesis, epidemiology and risk assessment, Toxicol. Sci. 120 (Suppl. 1) (2011) S130–S145.
[208] J. Cadet, K.J.A. Davies, Oxidative DNA damage & repair: an introduction, Free Radic. Biol. Med. 107 (2017) 2–12.
[209] B. Halliwell, O.I. Aruoma, DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems, FEBS Lett. 281 (1991) 9–19.
[210] M. Moriya, C. Ou, V. Bodepudi, F. Johnson, M. Takeshita, A.P. Grollman, Site-specific mutagenesis using a gapped duplex vector: a study of translesion synthesis past 8-oxodeoxyguanosine in E. coli, Mutat. Res. 254 (1991) 281–288.
[211] D. Wang, D.A. Kreutzer, J.M. Essigmann, Mutagenicity and repair of oxidative DNA damage: insights from studies using defined lesions, Mutat. Res. 400 (1998) 99–115.
[212] T. Umemura, S. Kai, R. Hasegawa, K. Sai, Y. Kurokawa, G.M. Williams, Pentachlorophenol (PCP) produces liver oxidative stress and promotes but does not initiate hepatocarcinogenesis in B6C3F1 mice, Carcinogenesis 20 (1999) 1115–1120.
[213] D.J. Loury, T.L. Goldsworthy, B.E. Butterworth, The value of measuring cell replication as a predictive index of tissue-specific tumorigenic potential Butterworth BE, in: T.J. Slaga (Ed.), Nongenotoxic Mechanisms in Carcinogenesis. NY: the Banbury Report, Cold Spring Harbor Laboratory, 1987, pp. 119–136.
[214] I.P. Pogribny, I. Rusyn, F.A. Beland, Epigenetic aspects of genotoxic and non-genotoxic hepatocarcinogenesis: studies in rodents, Environ. Mol. Mutagen. 49 (2008) 9–15.
[215] I.P. Pogribny, T. Kobets, F.A. Beland, Alterations in DNA methylation resulting from exposure to chemical carcinogens, eLS, John Wiley & Sons, Ltd, Chichester, 2014.
[216] R.A. Rollins, F. Haghighi, J.R. Edwards, R. Das, M.Q. Zhang, J. Ju, T.H. Bestor, Large-scale structure of genomic methylation patterns, Genome Res. 16 (2006) 157–163.
[217] D.J. Weisenberger, M. Campan, T.I. Long, M. Kim, C. Woods, E. Fiala, M. Ehrlich, P.W. Laird, Analysis of repetitive element DNA methylation by MethyLight, Nucleic Acids Res. 33 (2005) 6823–6836.
[218] Z. Herceg, P. Hainaut, Genetic and epigenetic alterations as biomarkers for cancer detection, diagnosis and prognosis, Mol. Oncol. 1 (2007) 26–41.
[219] S. Sharma, T.K. Kelly, P.A. Jones, Epigenetics in cancer, Carcinogenesis 31 (2010) 27–36.
[220] A.J. Bannister, T. Kouzarides, Regulation of chromatin by histone modifications, Cell Res. 21 (2011) 381–395.
[221] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[222] C. Koufaris, J. Wright, R.A. Currie, N.J. Gooderham, Hepatic microRNA profiles offer predictive and mechanistic insights after exposure to genotoxic and epigenetic hepatocarcinogens, Toxicol. Sci. 128 (2012) 532–543.
[223] M. Berdasco, M. Esteller, Aberrant epigenetic landscape in cancer: how cellular identity goes awry, Dev. Cell 19 (2010) 698–711.
[224] Y. Saito, G. Liang, G. Egger, J.M. Friedman, J.C. Chuang, G.A. Coetzee, P.A. Jones, Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells, Cancer Cell 9 (6) (2006) 435–443.
[225] M.V. Iorio, C. Piovan, C.M. Croce, Interplay between microRNAs and the epigenetic machinery: an intricate network, Biochim. Biophys. Acta 1799 (10–12) (2010) 694–701.
[226] T. Lindahl, My journey to DNA repair, Dev. Reprod. Biol. 11 (2013) 2–7.
[227] P. Modrich, R. Lahue, Mismatch repair in replication fidelity, genetic recombination, and cancer biology, Annu. Rev. Biochem. 65 (1996) 101–133.
[228] A. Sancar, Excision repair in mammalian cells, J. Biol. Chem. 270 (1995) 15915–15918.
[229] P.C. Hanawalt, Historical perspective on the DNA damage response, DNA Repair 36 (2015) 2–7.
[230] A.A. Larrea, S.A. Lujian, T.A. Kunkel, SnapShot: DNA mismatch repair, Cell 141 (2010) 730 e1.
[231] B. Sedgwick, P.A. Bates, J. Palk, S.C. Jacobs, T. Lindahl, Repair of alkylated DNA: recent advances, DNA Repair 6 (2007) 429–442.
[232] R. Goth-Goldstein, Repair of DNA damage by alkylating carcinogens is defective in xeroderma pigmentosum-derived fibroblasts, Nature 267 (1977) 81–82.
[233] N.K. Alvi, P.G. Foiles, G.M. Williams, Inhibition of repair of O6-methyldeoxyguanosine and enhanced mutagenesis in rat-liver epithelial cells, Mutat. Res. 230 (1990) 219–226.
[234] A.D. Thomas, G.J. Jenkins, B. Kaina, O.G. Bodger, K.H. Tomaszowski, P.D. Lewis, S.H. Doak, G.E. Johnson, Influence of DNA repair on nonlinear dose-responses for mutation, Toxicol. Sci. 132 (2013) 87–95.
[235] G.P. Margison, P. Kleihues, Chemical carcinogenesis in the nervous system. Preferential accumulation of O6-methylguanine in rat brain deoxyribonucleic acid during repetitive administration of N-methyl-N-nitrosourea, Biochem. J. 148 (1975) 521–525.
[236] J.E. Cleaver, Repair replication of mammalian cell DNA: effects of compounds that inhibit DNA synthesis or dark repair, Radiat. Res. 37 (1969) 334–348.
[237] R.E. Rasmussen, R.B. Painter, Evidence for repair of ultra-violet damaged deoxyribonucleic acid in cultured mammalian cells, Nature 203 (1964) 1360–1362.
[238] C.A. McQueen, B.M. Way, G.M. Williams, Genotoxicity of carcinogens in human hepatocytes; application in hazard assessment, Environ. Mutagen. Toxicol. Appl. Pharmacol. 96 (1988) 360–366.
[239] G.M. Williams, Carcinogen induced DNA repair in primary rat liver cell cultures: a possible screen for chemical carcinogens, Cancer Lett. 1 (1976) 231–235.
[240] G.M. Williams, Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures, Cancer Res. 37 (1977) 1845–1851.
[241] C.N. Martin, A.C. McDermid, R.C. Garner, Testing of known carcinogens and noncarcinogens for their ability to induce unscheduled DNA synthesis in HeLa cells, Cancer Res. 38 (1978) 2621–2627.
[242] V.A. Bohr, C.A. Smith, D.S. Okumoto, P.C. Hanawalt, DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall, Cell 40 (2) (1985) 359–369.
[243] I. Mellon, V.A. Bohr, C.A. Smith, P.C. Hanawalt, Preferential DNA repair of an active gene in human cells, Proc. Natl. Acad. Sci. U. S. A. 83 (23) (1986) 8878–8882.
[244] C. Lahtz, G.P. Pfeifer, Epigenetic changes of DNA repair genes in cancer, J. Mol. Cell Biol. 3 (2011) 51–58.
[245] T. Kouzarides, Chromatin modifications and their function, Cell 128 (2007) 693–705.
[246] A.D. Conti, T. Kobets, C. Escudero-Lourdes, B. Montgomery, V. Tryndyak, F.A. Veland, D.R. Doerge, I.P. Pogribny, Dose- and time-dependent epigenetic changes in the livers of Fisher 344 rats exposed to furan, Toxicol. Sci. 139 (2014) 371–380.
[247] E.R. Fearon, B. Vogelstein, A genetic model for colorectal tumorigenesis, Cell 61 (1990) 759–767.
[248] H. Land, L.F. Parada, R.A. Weinberg, Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes, Nature 304 (5927) (1983) 596–602.
[249] B. Vogelstein, K.W. Kinzler, Cancer genes and the pathways they control, Nat. Med. 10 (2004) 789–799.
[250] D. Jenssen, C. Ramel, Relationship between chemical damage of DNA and mutations in mammalian cells. I. Dose-response curves for the induction of 6-thioguanine-resistant mutants by low doses of monofunctional alkylating agents, X-rays and UV radiation in V79 Chinese hamster cells, Mutat. Res. 73 (1980) 339–347.
[251] A.D. Thomas, G.E. Johnson, DNA Repair and its influence on point of departure for alkylating agent genotoxicity, in: T. Nohmi, S. Fukushima (Eds.), Thresholds of Genotoxic Carcinogens – from Mechanisms to Regulation, Academic Press, NY, 2016, pp. 67–82.
[252] C.S. Potten, M. Loeffler, Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt, Development 110 (1990) 1001–1020.
[253] J.E. Till, Stem cells in differentiation and neoplasia, J. Cell. Physiol. Suppl. 1 (1982) 3–11.
[254] J.E. Trosko, Human adult stem cells as the target cells for the initiation of carcinogenesis and for the generation of "cancer stem cells, Int. J. Stem Cells 1 (2008) 8–26.
[255] J.L. Counts, J.I. Goodman, Alterations in DNA methylation may play a variety of

roles in carcinogenesis, Cell 83 (1995) 13–15.
[256] G.M. Williams, Liver carcinogenesis: the role for some chemicals of an epigenetic mechanism of liver tumor promotion involving modification of the cell membrane, Food Chem. Toxicol. 19 (1981) 577–583.
[257] G.M. Williams, K. Watanabe, Quantitative kinetics of development of N-2-fluroenylacetanide-induced altered (hyperplastic) hepatocellular foci resistant to iron accumulation and of their reversion or persistence following removal of carcinogen, J. Natl. Cancer Inst. 61 (1978) 113–121.
[258] W.R. Loewenstein, Junctional cell-to-cell communication and growth control, Ann NY Acad Sci 339 (1980) 39–45.
[259] L.P. Yotti, C.C. Chang, J.E. Trosko, Elimination of metabolic cooperation in Chinese hamster cells by a tumor promoter, Science 206 (4422) (1979) 1089–1091.
[260] A.W. Murray, D.J. Fitzgerald, Tumor promoters inhibit metabolic cooperation in cocultures of epidermal and 3T3 cells, Biochem. Biophys. Res. Commun. 91 (1979) 395–401.
[261] J.K. Chipman, A. Mally, G.O. Edwards, Disruption of gap junctions in toxicity and carcinogenicity, Toxicol. Sci. 71 (2003) 146–153.
[262] L.M. Kamendulis, J.S. Isenberg, J.H. Smith, G. Pugh Jr., A.W. Lington, J.E. Klaunig, Comparative effects of phthalate monoesters on gap junctional intercellular communication and peroxisome proliferation in rodent and primate hepatocytes, J. Toxicol. Environ. Health 65 (2002) 569–588.
[263] I.V. Budunova, G.M. Williams, D.C. Spray, Effect of tumor promoting stimuli on gap junction permeability and connexin 43 expression in the ARL 18 rat liver cell line, Arch. Toxicol. 67 (1993) 565–572.
[264] T. Lindahl, Instability and decay of the primary structure of DNA, Nature 362 (1993) 709–715.
[265] A.C. Povey, DNA adducts: endogenous and induced, Toxicol. Pathol. 28 (2000) 405–414.
[266] E.J. Calabrese, Hormesis: a fundamental concept in biology, Microb. Cell 1 (5) (2014) 145–149.
[267] W.J. Waddell, Comparisons of thresholds for carcinogenicity on linear and logarithmic dosage scales, Hum. Exp. Toxicol. 24 (2005) 325–332.
[268] W.J. Waddell, S. Fukushima, G.M. Williams, Concordance of thresholds for carcinogenicity of N-nitrosodiethylamine, Arch. Toxicol. 80 (2006) 305–309.
[269] H. Bartsch, R. Montesano, Commentary; relevance of nitrosamines to human cancer, Carcinogenesis 5 (1984) 1381–1393.
[270] D. Spiegelhalder, R. Preussmann, Occupational nitrosamine exposure. 1. Rubber and tyre industry, Carcinogenesis 4 (1983) 1147–1152.
[271] R.W. Hart, R.B. Setlow, Correlation between deoxyribonucleic acid excision-repair and life-span in a number of mammalian species, Proc. Natl. Acad. Sci. U. S. A. 71 (1974) 2169–2173.
[272] S.L. MacRae, M.M. Croken, R.B. Calder, A. Aliper, B. Milholland, R.R. White, A. Zhavoronkov, V.N. Gladyshev, A. Seluanov, V. Gorbunova, Z.D. Zhang, J. Vijg, DNA repair in species with extreme lifespan differences, Aging (Albany NY) 7 (2015) 1171–1184.
[273] Sender R, Fuchs S, Milo R., Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 14 (8) (2016) e1002533, https://doi.org/10.1371/journal.pbio.1002533.

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Mini-review

Dose-dependence of chemical carcinogenicity: Biological mechanisms for thresholds and implications for risk assessment

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ABSTRACT

Current regulatory practices for chemical carcinogens were established when scientific understanding of the molecular mechanisms of chemical carcinogenesis was in its infancy. Initial discovery that DNA mutation was the root of cancer led quickly to regulatory processes that assumed such a simple relationship could be described with a linear approach. This linear, no threshold approach has since become the default approach to risk assessment of chemicals with carcinogenic potential. Since then, a multitude of intrinsic processes have been identified at the molecular, cellular and organism level that work to prevent transient DNA damage from causing permanent mutations, and mutated cells from becoming cancer. Mounting evidence indicates that these protective mechanisms can prevent carcinogenesis at low doses of genotoxic chemicals, leading to non-linear dose-response. Further, a number of non-genotoxic mechanisms have demonstrated threshold-shaped dose-response for cancer outcomes. The existence of non-linear dose-response curves for both non-genotoxic and genotoxic chemical carcinogens stands in stark contrast to the default risk assessment approach that assumes low dose linearity. In this review, we highlight some of the key discoveries and technological advances that have influenced scientific understanding of chemical carcinogenesis over the last fifty years and provide case studies to demonstrate the utility of these modern technologies in providing a biologically robust evaluation of chemical dose-response for cancer risk assessment.

1. Historical perspective: scientific understanding and regulatory policy regarding chemical carcinogenicity

Any discussion of the potential to reform cancer risk assessment must be prefaced with the fact that current regulatory practices for chemical carcinogens were established when scientific understanding of the molecular mechanisms of chemical carcinogenesis was in its infancy. Initial discovery that DNA mutation was the root of cancer led quickly to regulatory processes that assumed such a simple relationship could be described as a linear dose response. This linear, no threshold approach has since become the default approach for risk assessment of chemicals identified to have genotoxic activity and/or carcinogenic potential, i.e., the default linear approach.

In the last few decades, much has been learned about the progression from initial DNA insult to carcinogenesis that challenges the validity of this default linear approachsnl n. A multitude of intrinsic processes at the molecular, cellular, and organism level work to prevent transient DNA damage from becoming a permanent mutation, mutated cells from forming tumors, and tumor cells from metastasizing. At the cellular level, these processes include post-translational DNA damage repair processes and transcriptionally regulated response pathways including DNA damage repair, cell cycle arrest, senescence and apoptosis. At the tissue and organism level, defenses such as cell-cell contact inhibition of proliferation and immune response to invading cancer cells must be overcome to develop cancer [1]. In the case of chemical-induced carcinogenesis, intrinsic protective mechanisms, including regulation of cellular redox levels via free radical scavengers, deactivation of reactive chemicals via phase II metabolism, and intrinsic repair processes that utilize nuclear proteins, have the potential to prevent propagation of DNA damage to mutation at low doses.

Results of studies with several genotoxic chemicals have demonstrated dose-dependent thresholds for mutation and genotoxicity in vivo and in vitro [2–6]. Fig. 1 shows an example of genotoxicity (measured as micronucleus frequency) in cultured human cells. While the responses to some chemicals are indistinguishable from linear, no threshold response at low doses (e.g., etoposide, Fig. 1A), others demonstrate an apparent threshold – or bi-linear (zero slope at low doses, positive slope at higher doses) – response (e.g., methyl methanesulfonate; Fig. 1B). Further, a number of in-depth assessments of chemical induced cancer have provided strong evidence for non-linear - or threshold-like - dose-response for cancer incidence curves [3,9–11]. In other words, the shape of the dose-response curve for chemical carcinogenesis may be more complex than the default assumption of linear, no threshold behavior – a fact that would dramatically affect estimated

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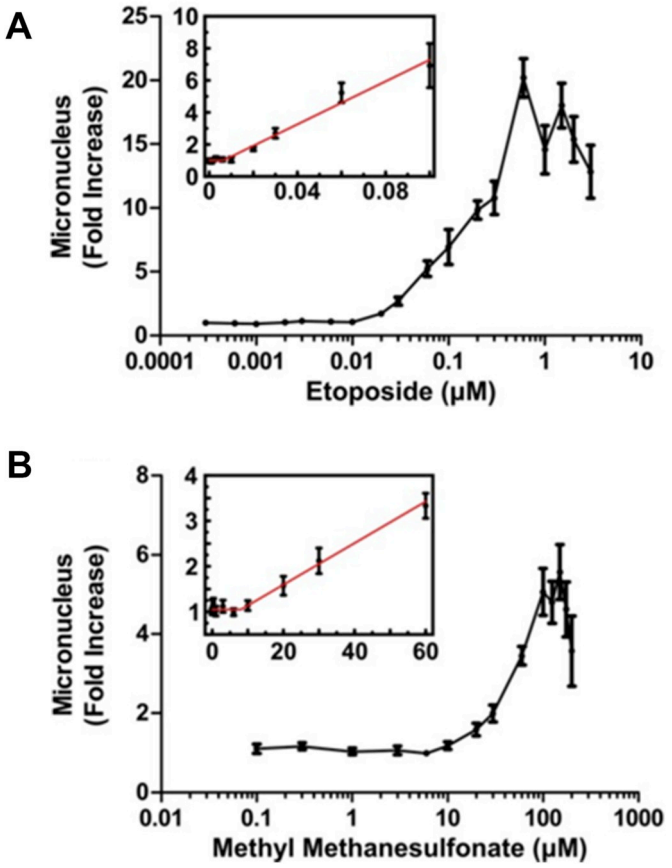

Fig. 1. Genotoxic response to two DNA damaging compounds in cultured human cells. HT1080 human fibrosarcoma cells exposed to (A) etoposide (topoisomerase II inhibitor) or methyl methane sulfonate (DNA methylating agent). The numbers of micronuclei per parent cell relative to vehicle controls (mean ± SEM) are plotted against compound concentration. Each concentration response was tested against the Lutz threshold model [7]. The red line in the inset shows the best fit bi-linear model. Only methyl methanesulfonate (panel B) demonstrated a statistically ($p < 0.05$) better fit to the data than a linear, no threshold model. Figure adapted from Ref. [8].

points of departure for risk assessments as well as the fundamental understanding of cancer risk (no added risk below the threshold vs. added risk at any dose).

In the following discussion, we briefly highlight some of the key discoveries and technological advances that have influenced scientific understanding of chemical carcinogenesis in light of their influence on perception of chemical risk and regulatory policies. In later sections of the paper, we provide case studies on the use of modern technology to better define the mechanisms that drive the dose-response curves for mutation and cancer, as well as various data streams that contradict the assumption that cancer is inherently a linear, no threshold (LNT) process.

1.1. Pre-1970 – early understanding of cancer and regulatory actions

In 1175, Sir Percival Pott hypothesized that the high rate of scrotal cancer in chimney sweeps was a result of their high levels of exposure to soot [12]. The near elimination of scrotal cancer with implementation of a regular bathing regimen lent powerful support to his hypothesis. By the early 20th century, mounting epidemiological evidence for associations between cancer and radiological or chemical exposures resulted in general acceptance of the possibility that environmental exposures could induce human cancers. In 1937, President Franklin D. Roosevelt signed into law the National Cancer Act, establishing the National Cancer Institute with the goal of improving understanding of the cause, diagnosis and treatment of cancer and a similar act was implemented in England in 1939. In 1944, Avery and coauthors identified DNA as genetic material [13] and Watson and Crick published a description of the chemical structure of DNA in 1953 [14]. The link between DNA and chemical carcinogenesis was quickly recognized, with discovery of DNA methylation by N-nitrosamines [15] and DNA adduct formation by aflatoxin B1 [16] and benzo[a]pyrene [17].

1.2. 1970s-1980s: first risk assessments for chemical carcinogens and development of the linearized multi-stage (LMS) cancer model

By the 1970s, it was clear that some chemicals could cause cancer in animals and humans: Cigarette smoking had been linked to lung cancer; asbestos was associated with pleural mesothelioma, and vinyl chloride was found to cause a rare liver tumor in experimental animals and in humans exposed occupationally. In the U.S. in particular, there was a rapid downhill spiral to public chemophobia fueled by a succession of highly publicized toxic chemical concerns: DDT, saccharin, FD&C Red No. 2, cyclamates, ethylene dibromide, dioxin, and Alar (on apples) [18]. In 1971, President Richard Nixon signed into law the National Cancer Act of 1971, aimed at strengthening the National Cancer Institute and increasing funding to cancer research.

Prior to this time, chemical risk assessments were conducted by identifying a No Observed Adverse Effect Level (NOAEL) in an experimental animal or human epidemiology study and applying uncertainty factors (UFs) addressing biological differences between experimental animals and humans and across individual humans to obtain an estimate of an acceptable exposure. The emergent development of a separate risk assessment methodology for chemical carcinogens, however, was driven by concerns that, due to the nature of the cancer process and associated limitations in the statistical power of experimental animal tests to detect chemically-induced cancers, even doses of a carcinogen well below those observed to induce tumors in animals could entail an unacceptable risk of cancer to humans. This assumption was augmented by the US National Academies of Science conclusion in 1956 that low-dose radiation exposures were presumed to cause cancer without a threshold because of their assumed potential to cause DNA damage and mutagenicity at any exposure level. Given that radiation- and chemically-induced genotoxicity were assumed to be essentially indistinguishable as key drivers of environmental carcinogenesis, this linear, no-threshold dose-response expectationfor carcinogenesis was considered inconsistent with the existing NOAEL/UF approach for chemical agents. As a result, low-dose extrapolation approaches were developed, culminating in the development of the “Linearized Multistage” (LMS) model [19], which used a statistical analysis to estimate the highest potency in the low-dose region that was statistically consistent with data on tumor incidence in an animal bioassay. The LMS model gained wide acceptance by regulators because it made it possible to obtain highly conservative estimates of cancer risks from low-dose exposures.

The first instance of a U.S. regulatory agency conducting a formal quantitative risk assessment (i.e., the calculation of a probability of harm) occurred in 1973, when a U.S. Food and Drug Administration regulatory document, “Compounds Used in Food-Producing Animals” (38 Fed. Reg. 19226, 1973), specified the required sensitivity of methods for measuring trace levels of carcinogens in meat products on the basis of the “maximum exposure resulting in a minimal probability of risk to an individual (e.g., 1/100,000,000) …. " (1 in 100 million.) A few years later, the 1980 U.S. Supreme Court decision on benzene provided the first clear mandate for quantitative low-dose extrapolation. Referring to OSHA's responsibility to protect workers from significant risk, the Court stated:

“It is the Agency's responsibility to determine in the first instance what it considers to be a “significant” risk. Some risks are plainly acceptable and others are plainly unacceptable. If, for example, the odds are one in a billion

that a person will die from cancer by taking a drink of chlorinated water, the risk could clearly not be considered significant. On the other hand, if the odds are one in a thousand that regular inhalation of gasoline vapors that are 2% benzene will be fatal a reasonable person might well consider the risk significant and take the appropriate steps to decrease or eliminate it." (I.U.D. v. A.P.I., 448 U.S. at 655).

A few years later, following widespread criticism of several risk assessment decisions made by health regulatory agencies, the U.S Congress commissioned a report by the National Academy of Science, "Risk Assessment in the Federal Government: Managing the Process," [20] that laid a formal foundation for modern chemical risk assessment. With chemical carcinogens, the Carcinogen Assessment Group (CAG) at the EPA developed tools for dose response assessments where any exposure, no matter how small, carried some probability of risk (Fed Regist, 41:21403, 1976). The basis of this approach – the linear, no threshold (LNT) approach – was developed based on studies of cancer induced by high doses of ionizing radiation [21], and a relatively new understanding that chemicals cause cancer through interaction with DNA. The processes governing DNA damage, DNA repair, prevention of heritable mutations and organism level responses to cancer were largely undiscovered at that time.

This early and relatively crude understanding of cancer biology served as the scientific basis for the development of the initial USEPA cancer risk assessment approach [22], and the resulting USEPA [23] guidelines specified a default assumption that low-dose risk is always linearly related to the dose and that any dose, no matter how small, poses some level of risk. The process developed for chemical carcinogens included low dose extrapolation with the LMS model and interspecies extrapolation from animal data to derive a slope factor. The LMS model was adopted as the statistical technique for providing a quantitative upper-bound estimate of risk from results of animal studies or epidemiological observations [24]. Other aspects of evaluating cancer risks included applying a surface area adjustment to convert animal-derived points of departure to equivalent human doses. The resulting slope factor relates risk and dose rate in units of $(mg/kg/day)^{-1}$. Multiplying this slope factor by the human exposure (mg/kg/day) and the number of people exposed provides an upper bound estimate for the expected lifetime increase in cancer incidence for the exposed population. Risk management practices were established to keep the calculated increase in increased risk below 1/1,000,000 (one per million) exposed individuals.

There was also an explosion in the 1970's and 1980's of new experimental methods to examine the consequences of lifetime exposures of rats and mice to chemicals and evaluate mutational properties in a wide array of assays. There was quickly a proliferation of compounds that were reported to cause DNA-damage or mutation in one or another assay, as well as compounds that were associated with increased cancers in in rats or mice, in studies that often used experimental doses that were far above those encountered in real-world human exposures in order to overcome limitations in statistical power.

1.3. 1990s-2000s: continued regulatory reliance on the LMS model despite improved understanding of the role of cell proliferation in carcinogenesis and development of biologically based dose-response models

By the late 1970s, chemical carcinogenesis was widely accepted to be a multistage process involving both mutation and cell division, both of which could be influenced by chemical dose [25]. Yet, there were no widely accepted dose-response models for cancer endpoints that accounted for the roles of both mutation and cell proliferation. The first efforts to address this deficiency were the development of clonal growth cancer modeling approaches by Moolgavkar [26] and colleagues. These models were initially developed for the interpretation of human epidemiological data on cancer incidence [27,28], but were subsequently applied for describing time-courses for both pre-neoplastic and neoplastic lesions in animal studies [29]. Clonal growth models of cancer

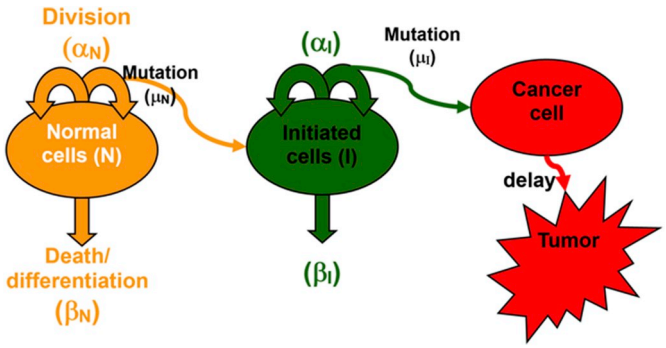

Fig. 2. Diagram of a 2-Stage Clonal Growth Cancer Model. In the 2-stage clonal growth model, the progression of normal cells (N) to fully neoplastic cancer cells is described as a series of two irreversible, mutation-driven events (μ_N and μ_i) with the possibility of clonal expansion of the intermediate stage. (α_N, α_i: cell division rates for normal/initiated cells; β_N, β_i: death/differentiation rates for normal/initiated cells.

provide a biologically plausible, mathematically rigorous framework for describing a nonlinear carcinogenic process. In a clonal growth model, the progression of normal cells to fully neoplastic cells is described as a series of at least two irreversible, mutation-driven events with the possibility of clonal expansion of the intermediate stage(s). The most commonly used model has 2 stages and is not meant to represent the detailed biochemical mechanism of most cancers. Nonetheless, the 2-stage clonal growth model (Fig. 2) is a particularly useful biologically motivated model of cancer since it is the simplest (most parsimonious) cancer model that allows for the incorporation of data on both cellular proliferation and mutation.

Importantly, the implications for tumor dose-response of separate dose-responses for cellular proliferation and mutation can be studied with the 2-stage model [30] to evaluate EPA concerns regarding the implications of "lurking genotoxicity" at low exposures to a chemical that causes both genotoxicity and cytotoxicity at higher exposures. This possibility has driven extensive investigation of the applicability of the 2-stage model to chemical carcinogens [31–33]. The advantages of linking pharmacokinetic models of DNA adduct formation to 2-stage clonal growth models of mutation and proliferation are described in a seminal paper by Cohen and Ellwein [34] that provides several case studies. One of the first instances where this approach was formally considered by an agency for use in a risk assessment was a model of formaldehyde nasal carcinogenicity developed by Conolly et al. [35,36]. This effort is discussed later in this paper as a case study on the use of mechanistic dose-response models and transcriptomic studies to demonstrate threshold shaped dose response, not only for the tumor response in animal bioassays, but also by extension to the cancer dose response for human formaldehyde exposures.

During this same time, researchers were able to clarify the relationship between DNA adduct formation and the potential for DNA mutation [37]. For mutation to occur, cell division must take place before the cell is able to restore the integrity of the damaged DNA. An important distinction was made that while DNA mutations can serve as biomarkers of effect, DNA adducts should only be considered biomarkers of exposure. That is, whereas the dose-response for mutations may be useful as a biomarker of effect as a precursor for carcinogenicity, the dose response for adducts should only be used as a surrogate for the intracellular concentration of the chemical (or its metabolite) reacting with DNA. They concluded that;

- *"biomarkers of exposure [i.e., DNA adducts] are usually linear at low doses, with the exception being when identical adducts are formed endogenously."*
- *"Whereas biomarkers of exposure extrapolate down to zero, biomarkers of effect [i.e., mutations] can only be interpolated back to the*

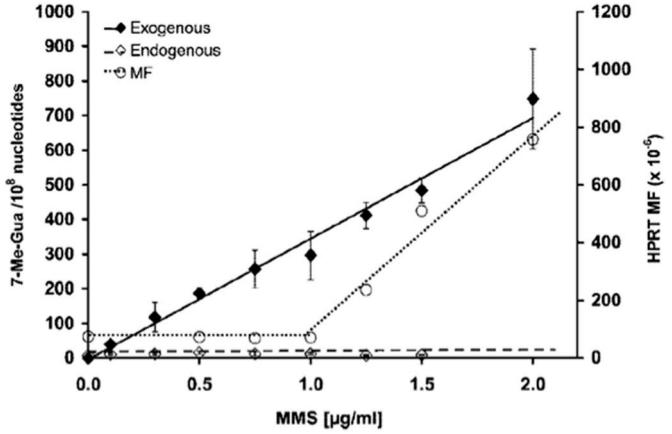

Fig. 3. Comparison of N7-methylquanine DNA adducts and HPRT mutations in AHH-1 cells exposed to MMS for 24 h. The endogenous adducts are N-7MeG (◇), while the exogenous adducts are [$^{13}C^{2}H_3$]-7Me-G (◆). The Hprt mutant frequency is shown as ◯. Figure reproduced from [37].

spontaneous or background number of mutations"

The implications of this distinction are illustrated in Fig. 3 (reproduced from Ref. [37], which shows the dose-responses for methane methyl sulfonate (MMS).

In response to the remarkable increase in understanding of the carcinogenic process during the 1980s and 1990s, the USEPA [38] updated their cancer risk assessment guidelines to provide for multiple options for carcinogen dose-response assessments driven by mode-of-action considerations. This represented a major departure from the previous USEPA [23] approach for cancer risk assessment. Important features include:

- Definition of default approaches as "no-data" options, the use of which must be justified based on the lack of sufficient information to support a more chemical-specific approach;
- Explicit support for biologically-based modeling as the preferred method for dose-response assessment;
- Definition of multiple low-dose extrapolation defaults: linear no threshold, nonlinear, and margin of exposure ("threshold"); and
- Consideration of the mode of action (carcinogenic mechanism) of the chemical both for determining the conditions under which the chemical should be considered a cancer hazard for humans, and for determining the appropriate low-dose extrapolation approach.

Under the new guidelines, mode of action considerations dictate whether a linear, no threshold dose-response assessment should be performed to derive a cancer potency estimate, or whether there is sufficient evidence of a highly nonlinear dose-response to justify the use of a nonlinear approach or harmonized toxicity value derivation. Harmonization of the cancer and noncancer risk assessment approaches has received widespread support [39] and was used in the USEPA [40] risk assessment for chloroform. In that assessment, a point of departure of 23 mg/kg/day was calculated based on the kidney tumor incidence in a drinking water study [41]. Comparing the point of departure to the agency's Reference Dose (RfD) of 0.01 mg/kg/day for non-cancer effects resulted in a Margin Of Exposure of 2,000, which was considered sufficiently large. Thus, in this case, the RfD for noncancer effects of chloroform was also considered adequately protective of public health for cancer effects, on the basis of the nonlinear dose response for chloroform and the mode of action for both cancer and noncancer effects having a common link through cytotoxicity.

Another rationale for a departure from linearity in the low-dose region comes from evidence for hormetic (J-shaped) dose-response behavior for toxicity [42], as well as for biomarkers of carcinogenicity [43] in animal studies. These observations raise the possibility that exposure to low concentrations of chemical carcinogens could actually decrease, rather than increase, cancer risk. There is still much debate in the risk assessment communities surrounding the possibility of unambiguously demonstrating threshold- or J-shaped dose-response curves. Some concerns are a result of uncertainty in sensitivity of the assays or the fact that few in vitro studies account for metabolism (bioactivation or inactivation). Other criticisms center around statistical analyses and the ability to fit a linear curve to the available data. In 2009, Lutz and Lutz developed a statistical approach to improve consistency in evaluating the likelihood of the existence of a threshold shaped dose-response [7]. This approach evaluates two alternative mathematical models against the data: a linear, no threshold model and a model that defines a threshold dose, below which the concentration response is flat, and above which the concentration is linear (i.e., a "hockey stick"). The two models are compared using the F-test, balancing goodness-of-fit against the number of parameters. While this model represents an important step forward in creating a standardized test for thresholds, there are challenges in interpreting the results of the Lutz model: the result are highly dependent on the statistical power of the experiment and only two possible models (linear, no threshold; hockey-stick) are compared. For these reasons, the authors provide the following word of caution in the relying purely on statistics to evaluate thresholds: "If the hockey stick model fits the data significantly better than linearity, the threshold-like appearance of the dose–response curve will have to be corroborated by mechanistic considerations and experimental testing of the respective hypothesis." [7]. Thus, to truly define the shape of the dose-response curves, we must look to the underlying biology and develop targeted experiments to identify and measure the key processes governing dose-response at low exposures.

1.4. 2010s: transcriptomics, the shift toward non-animal methods in toxicology, and pressure for regulatory agencies to accept new methodologies

Transcriptomics. When gene array technologies were introduced in the 1990's, the expectation was that these whole genome test methods would change the paradigm of life science research, including toxicology and its application to risk assessment. However, it was quickly recognized that the experimental technology had dramatically surpassed our ability to store, process, analyze and interpret such large datasets [44]. In the intervening years, the experimental technologies for transcription have evolved from RT-PCR for individual genes, to printed oligonucleotide arrays, to high throughput multiplexed RNA sequencing. Databases, computational and statistical methods, and methods for analysis and interpretation of transcriptomic data have also undergone a revolution [44]. Only recently have government and regulatory agencies begun to develop standardized approaches to the use of these data streams in chemical safety assessments.

Two particularly important advances in transcriptomic data interpretation for toxicological evaluation have been development of 1) gene ontology databases to support gene set enrichment analysis (or pathway analysis) and 2) development of a standardized approach to transcriptomic dose-response analysis based on gene set enrichment and traditional benchmark dose (BMD) principles. Pathway analysis helps to understand or interpret omics data in terms of canonical knowledge about biological processes using gene ontology databases such as Gene Ontology, KEGG, Ingenuity, Reactome or WikiPathways or by training predictive models using chemicals with known modes of action [45–47]; www.wikipathways.org, [5,48]. Recent work also demonstrated improved predictivity of transcriptomic signatures for apical response when evaluated in terms of pathways (i.e., suites of related genes) rather than as individual, unrelated genes [49]. The application of this technology is hindered, however, by the fact that the gene ontology databases are quite limited in their coverage of biology and in the completeness of the networks, which are confined by the experimental design of the studies that provide the underlying data.

The process of developing comprehensive networks that can be used to predict chemical mode of action is ongoing. However, as these methods evolve, so too will our understanding of chemical dose-response. Examples provided in the case studies below for formaldehyde, nickel and dioxin (TCDD) demonstrate how transcriptomics technologies are already contributing to efforts to define the mechansims of threshold-shaped dose-response for carcinogens.

The second breakthrough in transcriptomic analysis for toxicological assessment, is the application of BMD methods to genomic dose-response. BMD, which has been a trusted tool for decoding dose response for risk assessment, was recently applied to transcriptomic data [50,51]. A number of studies have since demonstrated that transcriptional points of departure following short term exposures (< 14 days) are predictive of the doses (points of departure; PoD) causing both non-cancer and cancer chronic toxicity, including tumor incidence after life-time exposures in rodents [49,51–53]. Further, the suite of genes affected by a compound can yield essential information about the toxicological response, suggesting mechanisms that can then be tested with targeted mode of action studies. Toxicogenomic responses have been successfully coupled to in vivo and in vitro model systems to identify mechanisms and categorize responses to many chemical stressors, including carcinogens [54–56]. A powerful potential outcome of this work is the ability to predict safe chemical exposures, and chemical mode of action, from short-term in vivo studies – potentially eliminating the need for chronic, high dose toxicity assays including the 2 year bioassay that has long been considered the gold standard for carcinogens.

Unfortunately, few risk assessments have taken advantage of transcriptomic data streams. A notable exception to this rule is the recent "Opinion on Scientific Evaluation of Occupational Exposure Limits for Nickel and its Compounds" from the European Chemicals Agency (ECHA) Risk Assessment Committee [57], which cited studies on the dose-response for transcriptional changes as a basis for their decision to assign nickel compounds an indirect mode of action with a threshold,

> "In vivo inhalation studies by Efremenko et al. [58,59] in rats show that transcriptional pathways affected by nickel subsulfide and nickel sulfate primarily reflect responses to toxicity, including inflammatory and proliferative signaling. In the case of nickel subsulfide indications on the activation of the pathways related to DNA damage were seen only at the two highest dose level (0.11, and 0.44 mg Ni/m3) with a NOAEL of 0.06 mg Ni/m^3 after 1 month exposure and BMD10 for the activation of inflammatory pathways was 0.06 mg Ni/m^3 whereas for oxidative stress pathways it was 0.11 mg Ni/m^3. These results give confidence for an indirect genotoxic mode of action driven by chronic toxicity, inflammation and proliferation, leading to misreplication and the threshold based on inflammatory and cytotoxic effects."

New Approach Methods in Regulatory Science. Traditionally, toxicity testing has involved high-dose studies in experimental animals and mathematical extrapolation to predict effects of low dose exposure in humans from high dose studies in animals. This paradigm, established in the 1960s, has remained largely unchanged and is still the general practice for regulatory agencies. However, given the advances in biology, particularly the development of human cell lines, induced pluripotent stem cells, and 3D organotypic in vitro model systems, there is a significant pressure to begin to incorporate non-animal methods into the practice of risk assessment. In 2007, the National Research Council (NRC) of the National Academy of Sciences released a report, "Toxicity Testing in the 21st Century: A Vision and a Strategy (TT21C)" (NRC, 2007), which called for a reorientation of toxicity testing. This new approach would focus on evaluating the responses of toxicity pathways (i.e., normal cellular signaling pathways that can be perturbed by chemical exposures) in well-designed assays using human cells in vitro to evaluate chemical safety. Further, the report stressed the utility of computational systems biology pathway models to define chemical dose-response based on intracellular dynamics, and the use of 21st century technologies, including high content imaging, transcriptomics, computational modeling (from statistical models to QSAR and machine learning approaches) to more accurately predict human risk.

This seminal report was an initial step in what has become a revolution in toxicological sciences. Over the last decade tremendous efforts have been made to develop and test the utility of in vitro and computational technologies for use in chemical risk assessment. Most of these efforts have focused on the utility of in vitro and computational approaches to prioritize chemicals for testing, rather than completely replacing animal testing, though the ultimate goal is use the New Approach Methodologies (NAMs) for quantitative risk assessments. Efforts such as ToxCast and Tox21 generate enormous amounts of in vitro screening data that have been used as the source material for innumerable computational approaches to evaluating chemical bioactivity. Recently, these in vitro bioactivity assessments, and computational models built from the data, have been incorporated into screening process for USEPA's Endocrine Disruptor Screening Program for chemical prioritization. The Office of Pesticide Programs has begun accepting supporting data from a defined suite of in vitro assays (combined in an Integrated Approach to Testing and Assessment; IATA) as an alternative to in vivo skin sensitization assays [60,61].

In many ways, cancer toxicology was ahead of the field in development of alternative assays. Due to the long latent period of cancer, it was imperative to find fast screening methods to identify potential carcinogens without waiting for the traditional two year rodent bioassay. In the 1970's, Bruce Ames developed a DNA mutation assay in bacteria [62]. Over the years, as the mechanisms of cancer were better defined, other in vitro assays were developed both to improve early identification of carcinogens and to support mode of action analyses. Assays have been developed to address key events along the cancer continuum: DNA damage (single and double strand breaks, stalled replication forks, micronuclei), DNA adduct formation, DNA repair, sister chromatid exchange, and heritable mutations (HPRT reverse mutation, PigA mutation, BigBlue cell lines) [63–68]. However, these methods, while used widely for screening, are seldom used for dose-response assessments; two year in vivo bioassays are still the standard for regulatory decisions. Currently, efforts, such as the International Workshops on Genotoxicity Testing (IWGT) Working Group on Quantitative Approaches to Genetic Toxicology Risk Assessment (the QWG) have performed extensive analyses on the utility of in vitro genotoxicity methods to predict in vivo cancer studies. While the initial results are promising (i.e., positive correlation between in vitro and in vivo studies), more data that allow direct comparison of the test systems are needed [69,70]. Moving forward, reducing the need for long-term animal tests through replacement with alternative computational and in vitro approaches is the only realistic path to evaluating the thousands of untested chemicals currently in commerce.

2. Case studies in the utility of modern technologies to define chemical dose-response

2.1. Formaldehyde: biologically based dose-response (BBDR) models and transcriptomic profiling to elucidate threshold shaped dose responses

The USEPA (IRIS) defines a BBDR model as a "A predictive model that describes biological processes at the cellular and molecular level linking the target organ dose to the adverse effect." One of the first BBDR models formally considered by an agency for use in a risk assessment was a model of formaldehyde nasal carcinogenicity developed by Conolly et al. [35,36]. This extensive multi-faceted data collection and modeling effort conducted at the Chemical Industry Institute of Toxicology [71] characterized the mode of action for formaldehyde nasal carcinogenicity in the rodent to provide a basis for estimating risk of formaldehyde carcinogenicity in humans. Formaldehyde is a high

volume industrial chemical with many uses, and it is also a normal endogenous product of intermediary metabolism in mammals that is present in all tissues. However, chronic inhalation of formaldehyde resulted in nasal squamous cell carcinoma (SCC) in F344 rats at air concentrations of 6 ppm and greater [72,73]. Formaldehyde is genotoxic and mutagenic (at relatively high concentrations compared to human exposures), and inhalation leads to the formation of DNA-protein cross-links (DPX) in the nuclei of exposed cells [74,75]. However, formaldehyde also produces cytotoxicity and induces compensatory cytolethal-regenerative cellular proliferation (CRCP) at concentrations in the low ppm range in the nasal cavity of rats [73]. Conolly and colleagues [76] proposed that the mode of action for the carcinogenicity of formaldehyde was increased fixation of background mutations by cytotoxicity-induced cell proliferation (by increasing the rate of cell proliferation, DNA repair does not have time to occur before cell division), and described an approach using a 2-stage clonal growth model to evaluate the dose-response for this process. Understanding the role of proliferation was considered crucial because formaldehyde-induced nasal SCC have only been reported at concentrations of formaldehyde that are known to be cytotoxic (and proliferative) in rats, and formaldehyde exposures have not been reported to result in cytotoxicity in humans [77].

Formaldehyde is a highly reactive, water soluble gas that is taken up readily into the epithelial tissues of the nasal passages as it passes through the nose following inhalation exposure [78]. To assess the potential effect of nasal airflow on lesion location and severity, Kimbell and Subramaniam [78] developed an anatomically realistic three-dimensional computational fluid dynamics (CFD) model of airflow in the F344 rat nasal cavity, which provides the capability for high resolution predictions of regional flux of formaldehyde from the inhaled air into the adjacent tissue. These CFD models were used together with a pharmacokinetic model of formaldehyde metabolism and production of DPX to describe the observed nonlinear increases in DPX with increasing exposure to formaldehyde due to saturation of tissue metabolic clearance [79,80]. These models were able to predict the levels of nasal mucosal DPX in rats, rhesus monkeys, and humans as a function of exposure concentration, reducing the uncertainty in predicting human nasal DPX formation resulting from formaldehyde exposure.

Based on this work, a BBDR model for the respiratory tract carcinogenicity of inhaled formaldehyde in rats was developed by Conolly et al. [35] utilizing predictions for regional flux produced by the CFD model [78,81] and linked with dose-response data for two potential aspects of the mode of action for formaldehyde: (1) direct mutagenicity, represented in the model by low dose linear DPX formation, and (2) cytotoxicity-driven proliferation, represented by nonlinear dose-response data for cell proliferation rates [73,74]. DPX formation had previously been used as a biomarker of tissue formaldehyde concentrations in cancer risk assessments for formaldehyde; however, Conolly et al. [35] used DPX formation as a surrogate for possible promutagenic lesions (i.e., the increase over the background probability of mutations that lead to SCC development is assumed to be proportional to DPX formation). The dose-response for cell proliferation was based on unit length labeling indices reported over a period of 6 weeks of exposure to formaldehyde by Monticello et al. [73,82] in six sites of the rat nasal passages, together with CFD model predictions of site-specific flux data for each region of the nasal airway. The BBDR model structure was based on the Moolgavkar [163] two-stage clonal growth model. The combined incidence of SCC in rat nasal passages reported by Kerns et al. [72] and Monticello et al. [73] along with historical control data for SCC reported by the National Toxicology Program were used to calibrate the model. The modeling indicated that the J-shaped dose-response for proliferation provided a better description of the SCC data than a linear, no threshold model; this analysis suggested that the rodent tumor responses were likely associated with a cytotoxic, rather than mutagenic, mode of action.

The conclusions of Conolly et al. [36] were that: "*the human implications of the rat squamous cell carcinoma (SCC) data indicates that (1) cancer risks associated with inhaled formaldehyde are de minimis (10-6 or less) at relevant human exposure levels and (2) protection from the non-cancer effects of formaldehyde should be sufficient to protect from its potential carcinogenic effects.*" Risk predictions derived from this model differed substantially from those obtained with models that relied more heavily on linear, no threshold defaults. For example, additional risk estimates due to lifetime exposure to 20 ppb ranged from 0 (J-shaped dose-response) to 9.7E-07 (hockey stick-shaped dose-response) based on the BBDR model [36] compared to 3.2E-04 based on the 1991 EPA unit risk factor (URF), a difference of at least 330-fold.

The dose dependent effects of formaldehyde in the nasal epithelium have also been evaluated using gene expression profiling of the target tissues in the anterior section of the rat nose [83]. New methods for analyzing dose-response microarray data using BMD modeling and gene ontology classification [50] were incorporated into an evaluation, in which concentration and exposure duration transitions in formaldehyde mode of action were examined using pharmacokinetic modeling for tissue formaldehyde acetal and glutathione together with the results of histopathology and gene expression analysis. Cell proliferation, histopathology, and gene expression in the nasal cavity were measured in rats exposed to concentrations of formaldehyde in air ranging from 0.7 to 15 ppm for 6 h a day over 1, 4, or 13 weeks [84]. The evaluation of nasal tissue responses in the rats showed significant increases in cell proliferation only at 6 ppm and above. There were no significant alterations of gene expression at the 0.7 ppm exposure, while genes associated with cellular stress, thiol transport/reduction, inflammation and cell proliferation were upregulated at 2 ppm. At 6 ppm and higher, gene expression changes showed enrichment of pathways involved in cell cycle, DNA repair, and apoptosis (Fig. 4).

These genomic results suggest that formaldehyde concentrations below 1 ppm would not increase the risk of cancer in the nose or affect formaldehyde homeostasis in epithelial cells. These results are consistent with the BBDR modeling results reported by Conolly et al. [35,36] suggesting a J-shaped dose response curve for formaldehyde and with the results of Kerns et al. [72] and Monticello et al. [73] that reported significant increases in the incidence of nasal squamous cell carcinomas following chronic inhalation exposure only at 6 ppm and greater.

The results of the formaldehyde BBDR modeling have been used to support risk assessment decisions by a number of regulatory agencies in the US and abroad, including the European Chemicals Agency [85], the Texas Commission on Environmental Quality (TCEQ) [164], and the European Union Scientific Committee on Occupational Exposure Levels [86]. While the USEPA [87] did not use the BBDR model in their risk assessment, the subsequent review by the National Academy of Sciences National Research Council [88] disagreed with the decision:

> "Given that the BBDR model for formaldehyde is one of the best-developed BBDR models to date, the positive attributes of BBDR models generally, and the limitations of the human data, the committee recommends that EPA use the BBDR model for formaldehyde in its cancer assessment, compare the results with those described in the draft assessment, and discuss the strengths and weaknesses of each approach".

This NRC recommendation to use the BBDR model as a basis for comparison of dose-response alternatives is consistent with the OMB [89] memorandum on risk analysis, which recommends the presentation of results from multiple dose-response approaches to provide a more robust risk characterization. In this scenario, the biologically based model can be used to determine the most scientifically plausible risk estimate for comparison with the results of alternative science-policy default approaches [90].

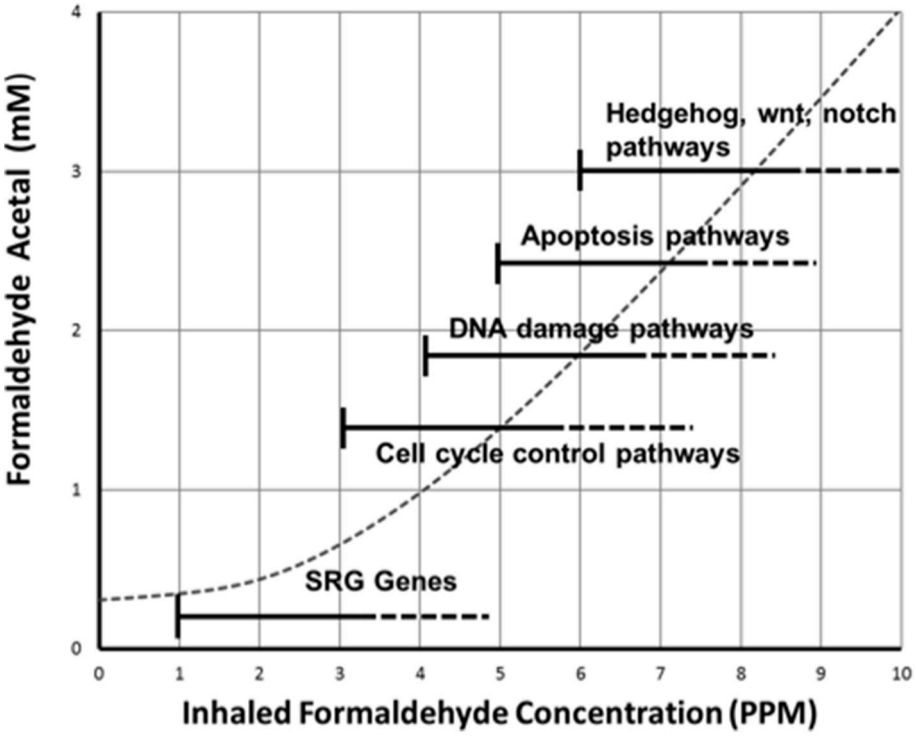

Fig. 4. Recruitment of toxicity pathway responses with increasing inhaled formaldehyde exposure. Figure reproduced from Ref. [84].

2.2. Hexavalent chromium

Until 2008, there was limited evidence of carcinogenicity of hexavalent chromium from oral exposure. The U.S. EPA's IRIS database listed toxicity criteria for Cr(VI) based on the absence of toxicity in rats exposed to up to 25 ppm for one year [91]. For reference, the mean and 95th percentile Cr(VI) concentrations in U.S. drinking water are 0.001 and 0.003 ppm [92,94,167]. In 2008, the National Toxicology Program conducted a 2-year cancer bioassay exposing F344 rats and B6C3F1 mice to 5–180 ppm Cr(VI) in drinking water [95]. At study termination, rats exposed to 180 ppm had increased incidence of oral cavity tumors, whereas mice exposed to ≥30 ppm had increased incidences of small intestine tumors. No tumors were observed in any other organs of either species. Given the dose-response for intestinal tumors and greater potency relative to oral cavity tumors, the intestinal tumors in mice have served as the basis for newly proposed oral toxicity criteria [96–101].

Despite clear indications that Cr(VI) caused chronic mucosal irritation and injury leading to significant life-time increases in crypt hyperplasia, several regulatory agencies have proposed toxicity criteria using the LNT concept, resulting in 10^{-6} cancer risk levels that equate to water concentrations as low as 0.00002 ppm (i.e. 0.02 ppb) [97], which is 50-fold lower than typical concentrations in U.S. drinking water. As part of a large industry-funded research program to better understand the mode of action for the intestinal cancers observed in mice at very high Cr(VI) concentrations, two specific technologies were instrumental in informing the mode of action and providing scientific justification for developing toxicity criteria that did not employ the LNT concept. One of these technologies provided critical dosimetry data, and the other provided critical biological response data. Together, the two technologies provide strong and corroborative evidence for a non-mutagenic mode of action for intestinal cancer in mice.

Similar to how dosimetry information has informed the mode of action of formaldehyde, advances in the development and use of dosimetry data have greatly informed mode of action analysis for Cr(VI). Metals such as chromium can be traced in biological samples using X-ray fluorescence (XRF) microscopy [102,103], allowing researchers to see which cell populations are exposed to the agent of interest.

In collaboration with scientists at the U.S. Army Engineer Research and Development Center (Vicksburg, MS) and Brookhaven National Laboratory (Upton, NY), synchroton-based XRF microscopy was used to examine the location of Cr in the small intestines of mice following exposure to 180 ppm Cr(VI) in drinking water for up to 90 days [104]. In transverse duodenal sections from mice exposed to 180 ppm Cr(VI) for 90 days, Cr was detected in the villous regions of the mucosa but not the crypt region (Fig. 5A–B). Intestinal crypts contain pluripotent stem cells that are responsible for generating the intestinal mucosa; these stem cells generate daughter cells that proliferate and migrate within the so-called transit amplifying region, and eventually become fully differentiated enterocytes of the intestinal villi [105,106]. It takes approximately three days for daughter cells to transit from the lower crypt to the villus tips and ultimately slough into the intestinal lumen [107]. Importantly, intestinal tumors are believed to originate from mutations in crypt stem cells [108].

Consistent with the absence of Cr in the crypt region, in vivo intestinal micronucleus assays were negative in mice exposed to Cr(VI) for 7 or 90 days [109]. In a separate study, mice were exposed to ≤180 ppm Cr(VI) in drinking water for 7 days and again, XRF maps indicated the presence of Cr in villi but not the crypts (Fig. 5C) [110]. In contrast to mice exposed to Cr(VI), mice exposed to the positive control cyclophosphamide exhibited signs of genotoxic insult: significant increases in micronuclei, karyorrhectic nuclei, and unusual staining of nuclear material with anti-phospho-H2AX antibodies in the crypt compartment [110]. The absence of genotoxicity in the crypt compartment carries the unequal burden of trying to 'prove a negative'; however, coupled with the XRF mapping these genotoxicity data are quite compelling. Specifically, negative genotoxicity results are to be expected if the agent (regardless of its genotoxic potency) does not reach the DNA of the population of cells at risk for being damaged and that proliferate.

Another important technology used to support a non-linear mode of action was the Big Blue® transgenic rodent (TGR) model. As mentioned previously, rats exposed to 180 ppm Cr(VI) via drinking water

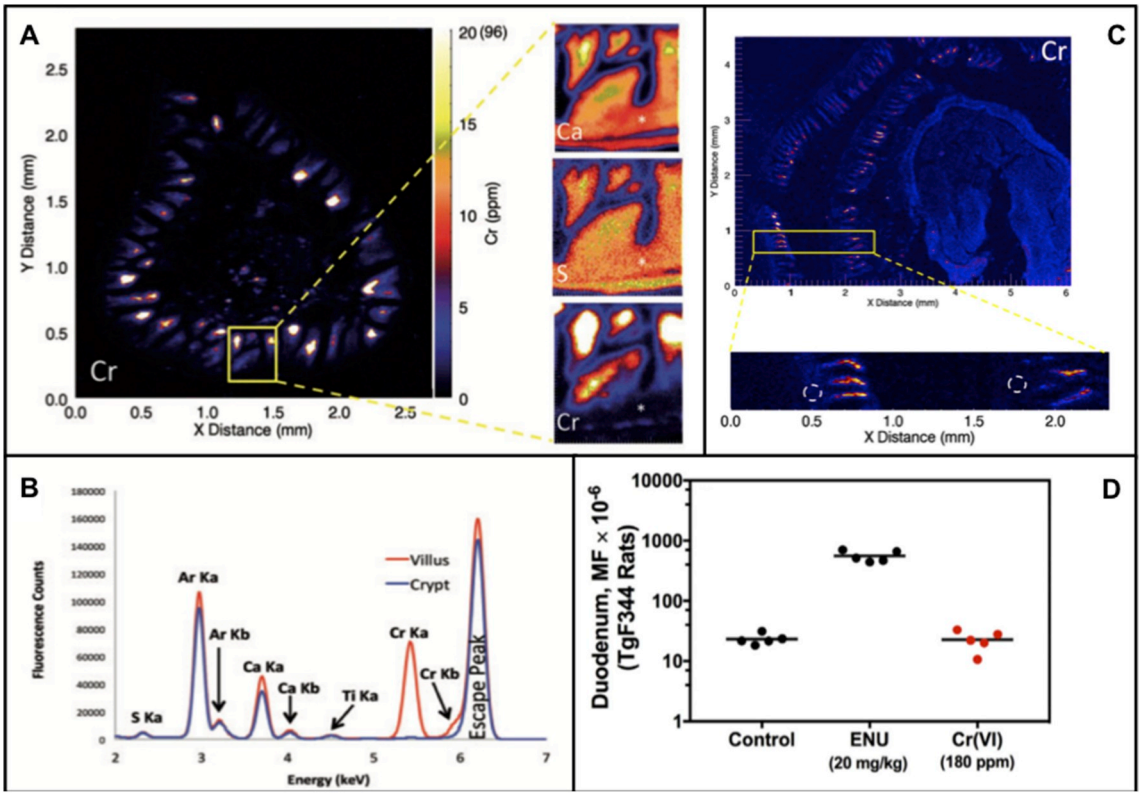

Fig. 5. The role of XRF microscopy and transgenic rodent mutation assay in investigating the mode of action for intestinal tumors in mice. A. XRF Cr map of a transverse duodenal section (left) from a mouse exposed to 180 ppm Cr(VI) for 90 days via drinking water. Magnified images (right) of crypt (asterisk) and villus signal in the same intestinal section. The stacked images from the same sample show an XRF calcium (Ca) map, XRF sulfur (S) map, and XRF Cr map. Note the striking absence of signal in the crypt region only in the XRF Cr map (magnified images are in different units from the main map on the left). B. Representative multichannel array plot depicting fluorescence (Ka and Kb) emission lines for the villus and crypt regions of a duodenal section from a mouse exposed to Cr(VI) for 90 days. C. XRF Cr map of a Swiss roll of duodenum from a mouse exposed to 180 ppm Cr(VI) in drinking water for 7 days. The white circles mark the crypt region. Note: the colors represent fluorescence signal intensity ranging from blue (low signal) to white (high signal). D. Mutant frequency (MF) in the duodenum of TgF344 rats (n = 5). The MF of 1-ethyl-1-nitrosourea (ENU) treated rats increased significantly ($P < 0.001$) relative to controls (1-way ANOVA).

developed oral cavity tumors. Studies into the mode of action for the oral tumors revealed no evidence of histopathological lesions or transcriptomic changes following exposure to up to 180 ppm Cr(VI) for 7 or 90 days [111,112]. To assess the potential involvement of direct mutagenicity in the rat oral cavity, the Big Blue® transgenic rodent in vivo mutation assay was used to evaluate two locations within the rat oral mucosa that were thought to be the origin of the oral tumors. These transgenic rodents have multiple copies of a shuttle vector containing bacterial and bacterial phage transgenes (lacI and cII) that can be extracted from most any tissue to assay for mutations that might have occurred in these 'reporter' genes in vivo [113]. After demonstrating that the assay could reliably detect in vivo mutations in both regions of the oral mucosa following exposure to 4-nitroquinoline-1-oxide [114], a TGR mutation assay based on OECD Test Guideline 488 [115] was conducted with Cr(VI). Big Blue rats (TgF344) rats exposed to the positive control (10 ppm 4-nitroquinoline-1-oxide) for 28 days had significant increases in mutant frequency (MF) in both regions of the oral mucosa, whereas TgF344 rats exposed 180 ppm Cr(VI) for 28 days exhibited no change in MF in either region [116].

Although rats did not develop tumors in the small intestine, it was previously shown via XRF mapping that rats exposed to 180 ppm Cr(VI) for 90 days, like mice, had Cr clearly present in duodenal villi but not crypts [104]. It was therefore determined that appropriately stored duodenal segments from the aforementioned Big Blue rat study could be analyzed to inform the mutagenic potential of Cr in the duodenum under the reasonable assumption that if the metal is mutagenic, it is likely mutagenic in both rats and mice. Duodenal samples from TgF344 previously exposed to 1-ethyl-1-nitrosourea for six days (study days 1, 2, 3, 12, 19, 26) exhibited a significant increase in mutation frequency (MF), whereas TgF344 rats exposed 180 ppm Cr(VI) exhibited no change in MF (Fig. 5D) [117].

Together, XRF microscopy and the TGR in vivo mutation assay in target tissues in species that develop tumors in cancer bioassays provide compelling evidence for a threshold mode of action for the oral carcinogenicity of Cr(VI). The TGR assay alone provides important data against the LNT default assumption, but negative results are almost always met with skepticism—even more so when studies are conducted by industry stakeholders. However, the XRF microscopy provides important components of the modified Hill criteria. First, it provides consistency, i.e. genotoxic assays should be negative (regardless of the genotoxic potential of the agent) if the agent does not reach critical cell populations. Second, it provides specificity. Cr was detected in the villi of both rats and mice, but only mice were reported to have extensive signs of the regenerative crypt hyperplasia that is hypothesized to lead to tumor development late in the study [118]. Finally, these data are supported by biological plausibility. Not only because increased cell proliferation is a known driver of cancer [119,120], but by other studies that inform intestinal biology. A study with benzo[a]pyrene found induction of CYP1A1 in intestinal villi (but not crypt) of Dlb-1 mice following oral exposure; whereas CYP1A1 induction was observed in the crypt (but not villi) following i.p. exposure [121]. These data indicate i) that CYP1A1 can be induced in intestinal crypt stem cells in response to benzo[a]pyrene, and ii) benzo[a]pyrene does not reach the crypts from the intestinal lumen. Consistent with these results, the number of mutated crypts in Dlb-1 mice were higher following i.p. injection than oral exposure to benzo[a]pyrene. Brooks et al. concluded, "[t]hese

observations show at least in respect of B[a]P that the crypt stem cells should be regarded as part of the systemic compartment and not of that compartment directly accessible to compounds present in the intestinal lumen." The lack of Cr in the crypts after 90 days of exposure suggests that Cr did not reach crypts from either the lumen or blood compartments.

In light of the above data, it should be noted that the lowest carcinogenic concentration (30 ppm) in the 2-year cancer bioassay is 30,000 times higher than levels found in typical U.S. drinking water, yet 180 ppm was not overtly toxic to rodents, not mutagenic, and not genotoxic. As one expert panel member reviewing the U.S. EPA draft Cr (VI) assessment commented, "Cr(VI) is at best a very weak "mutagen", requiring very high doses that kill most cells and experimental "backflips" to select for survivors, and (iii) what we thought was "mutagenesis" is actually selection for stochastic cell survivors of massive toxic insult" [122]. In the years since these and other mode of action studies have been published, several groups have proposed threshold toxicity criteria for the intestinal tumors in mice and discounted concern for the oral tumors observed in rats only at 180 ppm [96,99,100,123]. The Cr(VI) case study highlights the strengths of a multifaceted research project. Like other examples herein, transcriptomics and pharmacokinetic modeling have also played an important role in understanding the mode of action for Cr(VI). This case study has highlighted just two of the assays that have played a critical role in the overall research project (https://cr6study.info).

2.3. Dioxin

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a member of the class of structurally similar chlorinated hydrocarbons, chlorinated dibenzo-*p*-dioxins, also referred to as Dioxin-Like Compounds (DLCs). TCDD is found as a contaminant in commercially produced chemicals such as chlorophenoxy herbicides (e.g., 2,4,5-trichlorophenoxyacetic acid), and DLCs are produced inadvertently in paper and pulp bleaching, waste incineration, fossil fuel and wood combustion [124]. In 2001, TCDD was listed by the National Toxicology Program [125] as a "known human carcinogen" based on carcinogenicity in experimental animals and human epidemiological studies. However, this classification remains controversial [126,127]. In the original NTP rodent cancer bioassay, increased liver neoplasms were observed in female rats and male and female mice exposed to 0.05, 0.5, and 2 μg/kg/week [128]; increased thyroid follicular-cell adenomas were also observed in male rats and female mice. A follow-up bioassay in female Sprague-Dawley rats showed neoplastic effects in the liver, lung, oral mucosa, and uterus [129]. Based on genetic studies and structure activity relationships, the mode of action of TCDD is generally accepted in the scientific community to be nongenotoxic activation of signal transduction pathways by ligand-activated AhR [130–134]. The activation of the AhR by TCDD and its potential role in carcinogenesis has been extensively studied [135]; however, the exact signal transduction pathways responsible for many of the carcinogenic effects remain to be identified.

The initial USEPA *Health Assessment Document for Polychlorinated Dibenzo-P-Dioxins* identified TCDD as a probable human carcinogen but did not calculate a cancer risk [136]. The subsequent draft risk assessment for dioxins, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* [137], characterized TCDD as a nongenotoxic carcinogen and a potent promoter, and derived a cancer potency estimate of 1×10^{-3} per pg/kg/d based on epidemiological studies and animal bioassays. In developing these cancer potencies, USEPA [137] used a linear, no threshold model to derive estimates of the 1% effective dose (ED_{01}) and the lower 95% confidence limit on the ED_{01} (LED_{01}), where dose was based on body burden of TCDD and intakes corresponding to the ED_{01} and LED_{01} values were estimated by pharmacokinetic modeling. The National Academy of Science [138] published a review of the USEPA [137] draft risk assessment, *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment,* and noted several deficiencies, including the lack of consideration of the possibility of a nonlinear dose-response, the lack of an objective evaluation of mode of action of TCDD, and the lack of a quantitative uncertainty analysis.

Simon et al. [126] conducted a both a linear, no threshold and a nonlinear, threshold-based risk assessment approach for TCDD based on the more recent NTP [129] cancer bioassay, which observed increased incidence in several tumor type in female Harlan Sprague-Dawley rats. They derived a low-dose linear cancer potency estimate of 1×10^{-4} per pg/kg/d, a factor of 10 lower than the potency estimate in USEPA [137]. However, based on their determination that "the most likely hepatocarcinogenic mode of action for TCDD and other aryl hydrocarbon receptor (AHR) agonists involves tumor promotion of spontaneously initiated hepatocytes that occurs with threshold-dependent characteristics" [139]), they also calculated a RfD with a value of 100 pg/kg/d by applying uncertainty factors to BMD_{01} values estimated on the basis of rat internal and human external doses calculated with a toxicokinetic model. The RfD was 100 times higher than the 10^{-4} risk-specific dose (RSD) based on the linear cancer slope factor.

The USEPA [140] responded to the NRC [141] recommendations in the report, *EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments,* which was then reviewed by the agency's Scientific Advisory board [142]. The resulting USEPA SAB [142] report chided the agency for not being fully responsive to the NAS [138] recommendations, and identified "major deficiencies" in the USEPA [140] response with respect to several critical recommendations regarding the USEPA [137] assessment included in the NAS [138] review:

- *"The SAB finds that the Report did not respond adequately to the NAS recommendation to adopt both linear and nonlinear methods of risk characterization in order to account for the uncertainty of the dose-response curve for TCDD. The Report states that only a linear approach could be justified. We recommend that EPA revise the Report to provide a balanced discussion of evidence of possible modes of action, including linear and nonlinear approaches for cancer endpoint."*
- *"EPA's Report discusses a broad range of philosophical and methodological issues to be considered in conducting an uncertainty analysis for TCDD toxicity. The SAB does not agree with the argument that conducting a unified quantitative uncertainty analysis is unfeasible and we have suggested a number of methods that could be used for this purpose."*

As a result of the USEPA SAB [142] review, the USEPA withdrew the cancer risk assessment for dioxin and announced they would proceed only with the noncancer risk assessment [143]. Since then there has been no further action by the agency regarding the cancer risk assessment for TCDD.

Overall, the regulatory history for dioxin provides a cautionary tale for the difficulty of incorporating new scientific understanding into regulatory decisions that are, by policy, intended to be conservative from the perspective of health protection. After repeated recommendations from the SAB and the NAS to consider a nonlinear dose-response for dioxin, the USEPA [140] report still argued that only a linear, no threshold approach could be justified and therefore only derived two examples of using a nonlinear approach that they characterized as "an illustrative exercise only." Their determination that only a linear, no threshold approach could be justified was supported by a large amount of data related to the mode of action for the carcinogenicity of TCDD, but with an apparent bias towards presenting evidence that supported the use of a default linear approach rather than providing an objective evaluation of alternative mode-of-action hypotheses. The possibility of a nonlinear mode of action appears to have been described only to the extent necessary to be able to present arguments against it, ignoring the fact that the fundamentally nonlinear nature of the dose-response for receptor mediated processes underlies the conviction of a large segment of the scientific community that the risk assessment for dioxin should use a nonlinear approach. The

intransigence of the USEPA to repeated requests from the EPA's own SAB and the NAS to include a nonlinear risk assessment option for TCDD has undoubtedly contributed to the delay in completion of the cancer risk assessment for TCDD for more than a decade.

2.4. Using cellular models to define chemical mechanisms underpinning threshold shaped dose-response

Distinguishing linear, no threshold vs. non-linear dose-response using in vivo models is traditionally challenging due to limited animal numbers (sample sizes, number of doses evaluated), confounding factors with aging animals, and limitations in the inferences that can be drawn from apical endpoints, such as observed tumor incidence or survival. Further, while modern genomic and imaging tools are useful for better defining in vivo mode of action, targeted analysis of the response in the susceptible cell population is still limited by technology. However, over the last decade, the proliferation of tools and technologies for in vitro biology have opened up new avenues for evaluation of the molecular and cellular processes that drive chemical dose-response. High content imaging allows in-depth, quantitative evaluation of cellular morphology and cell-cell interactions in 2D and 3D systems (i.e., in vitro pathology). Live cell imaging allows visualization of DNA damage and repair - in real time. Transcriptomics of cell populations or even single cells can be used to evaluate the change in the cellular environment over dose and time. Mutation assays in mammalian cells allow quantitation of the first irreversible step toward cancer, and support comparison of very early chemical-molecular interactions (DNA binding, reactive oxygen species production, etc), with downstream cellular events (transcriptomic, epigenetic, and phenotypic changes). Assays that can be used to measure the key events in cellular response to DNA damage are shown in Fig. 6. With thoughtful experimentation, and dose-response modeling, we can identify and describe the quantitative relationships within cells that drive dose-response and provide biological underpinning for the shape of chemical dose-response curves.

In a series of studies, Doak and colleagues evaluated the mutation dose response for a series of alkylating agents: methylmethane sulfonate (MMS), methylnitrosourea (MNU), ethylmethane sulfonate (EMS), and ethylnitrosourea (ENU). Chromosomal damage and point mutations were quantified with the micronucleus and hypoxanthine phosphoribosyltransferase (HPRT) forward mutation assays [144,145]. Micronuclei are small pieces of chromosomes or whole chromosomes lost during mitosis due to clastogenic or aneugenic chemical exposure. Initial studies demonstrated an apparent threshold with MMS and EMS, but not MNU or ENU, with both the micronucleus and HPRT assays. However, follow-up studies with lower doses of ENU did demonstrate thresholds [4], demonstrating the importance of dose selection in defining low dose response. In later work evaluating the potential biological reason behind the apparent threshold behavior, Doak et al. [144] demonstrated that lower doses of MMS lead to upregulation of the demethylating protein O6-methylguanine DNA methyltransferase (MGMT) and no concurrent response in mutation frequency, while higher doses of MMS lead to reduced MGMT and increased mutation frequency. The authors concluded that MGMT is a key line of defense against mutation at low exposures to MMS. These studies highlight the importance of experimental design in evaluation of linear, no threshold vs. threshold-shaped responses, including the need for evaluation of low doses where responses are not expected. They also demonstrate the importance of demonstrating the biological underpinning of apparent observed threshold response. The need for biological support for apparent thresholds due to inherent uncertainties in the data anlayis techniques, was also emphasized by Lutz and Lutz in their seminal paper describing the “hockey stick” model for the analysis of threshold shaped dose response [7]. In an effort to apply this concept more broadly, we undertook a series of studies aimed at identifying biological mechanisms that contribute to threshold-shaped dose-response across carcinogens with different mechanisms [5,8,146,147]. These studies are described in brief below.

p53 mediated responses to DNA damage. p53, the so-called “guardian of the genome”, is activated in response to various types of DNA damage and functions in multiple ways to preserve genome stability; it acts as both a recruitment factor for extant nuclear DNA repair enzymes and a transcription factor to initiate cellular responses including cell cycle arrest, DNA repair, apoptosis and senescence [148–155]. Failure of these processes leads to heritable mutations – irreversible changes in the DNA that could increase the tumorigenic potential of the cell. While p53-independent mechanisms can prevent DNA damage and mutation at the cellular level - from inactivation of reactive oxygen species by antioxidants to p53 independent DNA repair and apoptosis - we focused our studies on p53 mediated cellular events because p53 is consistently activated following DNA damage-irrespective of chemical mechanism.

Defining assay endpoints and conducting dose-response evaluations.

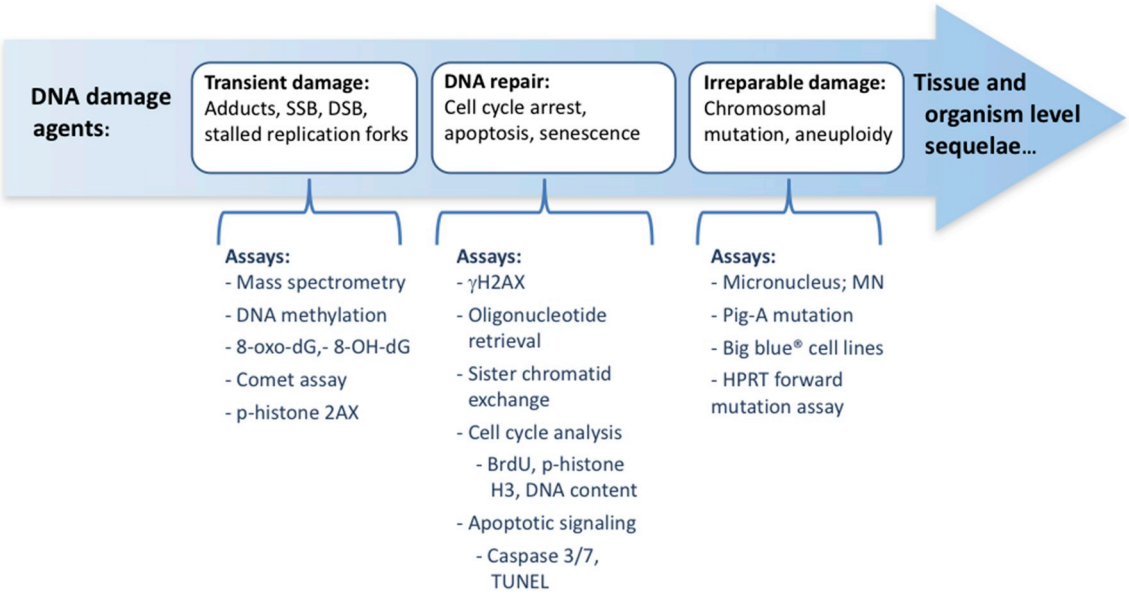

Fig. 6. DNA damage, cellular responses, and in vitro tests. Listed assays are examples of current technologies, and do not constitute an exhaustive list of available assays.

Initial focus was on defining the key readouts for the p53-mediated DNA damage response and conducting dose-response assessments using prototype DNA damaging chemicals [8,146,147,156]. Studies were performed in a human fibrosarcoma cell line (HT1080) that expresses wild-type p53 and follow-up confirmation studies were performed in the TK-6 cell line, a human lymphoblastoma cell line commonly used for genotoxicity testing. In-depth dose-response curves were generated for key aspects of DNA damage response, including: DNA damage (p-H2AX), p53 activation (p53, p-p53 (ser15)), cell cycle arrest (BrdU/pH3), apoptosis (caspase 3/7), and irreparable chromosomal mutation (micronuclei formation). Micronuclei are small pieces of DNA or whole chromosomes lost during mitosis following clastogen or aneugen exposure and were used as a surrogate for mutation endpoints (irreparable damage). We also performed whole genome transcriptomic dose-response studies. Three chemicals with different modes of action were used to probe the cellular response to different types of DNA damage: etoposide (ETP; topoisomerase II inhibitor and double strand break inducer); methyl methanesulfonate (MMS; methylating agent and single strand break inducer), and quercetin (QUE; oxidative DNA damage). The resulting data were then used to evaluate the shape of the dose-response curves for each of the measured biomarkers in an effort to identify the cellular processes (if any) that prevent micronuclei at low doses [8].

The original hypothesis was that cellular processes such as cell cycle arrest and apoptosis would prevent induction of permanent damage (micronuclei) at low doses. To test this, the endpoints listed above were measured across concentration and time, with 18 concentrations at a single time point shown to be a universally responsive in preliminary time course studies (24 h). Transcriptomic studies were performed at 24 h for 5 concentrations of each chemical. Surprisingly, when the dose-response trends were compared across endpoints, it was clear that many of the endpoints conventionally recognized as protective against DNA-damage —p53 protein accumulation, cell cycle arrest and apoptosis by p53 — occurred at higher doses than induction of micronuclei formation (Fig. 7). In fact, with all of the chemicals, micronuclei induction occurred at doses less than or equal to doses required to activate p53-mediated gene transcription (Fig. 7). Thus, any protective effect of p53 against micronuclei formation is unlikely to result from changes in transcriptional programs in the cells. Instead, it appears that the ability of p53 to prevent changes in the net level of permanent DNA damage at low chemical doses is likely due to post-translational processes, i.e., extant repair proteins at the site of DNA damage. This is consistent with in vivo studies demonstrating that doses that induced pathway level changes in gene transcription were very consistent with the doses leading to cancer in two year bioassays [49,51].

Common network motifs that maintain homeostasis in the presence of low chemical doses. Transcriptional up-regulation of stress response genes constitutes a major cellular defense program against a variety of chemical induced cellular stresses [166]. Cellular activation of transcriptional programs, however, requires significant time for RNA and protein synthesis and consumes considerable cellular energy stores. For many types of cellular stress (oxidative damage, DNA damage, heat and osmotic shock, *etc.*), post-translational processes also work to protect cells [157]. Post-translational responses are rapid and do not require transcriptional activation of genes. The rapid response by post-translational control can maintain cellular homeostasis in the presence of transient, low-level chemically-induced stresses and damage. With sustained or higher levels of damage, these post-translational processes become overwhelmed, forcing a transition to the slower responding, less efficient transcriptional controls. As a response to DNA damage, p53 plays a dual role (Fig. 8) – it acts (1) as a transcription factor, upregulating genes that initiate cell cycle arrest and apoptosis and (2) as a co-factor that helps to form DNA repair centers (DRCs), complexes of kinases, scaffold proteins and DNA repair enzymes that act directly on the DNA to repair damage [165]. These DRCs repair DNA damage without requiring activation of transcription, preventing long-term DNA damage.

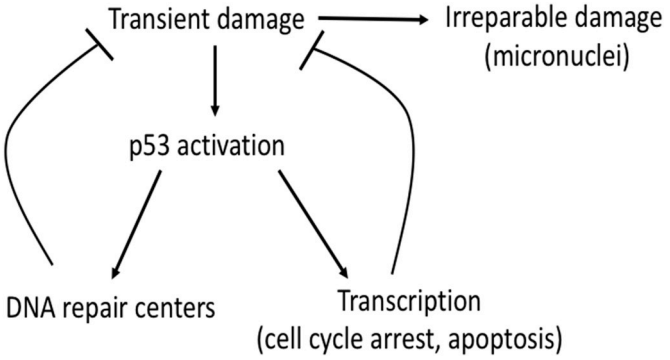

Fig. 8. p53 initiates post-translation and transcriptional responses to DNA damage. Transient damage leads to rapid activation of p53 and induction of repair centers (within minutes of initial damage), followed by p53-mediated transcriptional response (within hours of initial damage). Micronclei can be observed within a few hours of treatment in non-synchronized cultures. Standard in vitro procedures measure micronucleus induction at 1.5 cell cycle times to maximize response.

Defining regions of adaptive response and a point of departure for chemical safety assessment. As a proof of concept, neocarzinostatin (NCS) and etoposide were used to confirm the role of post-translational repair in preventing genotoxic outcomes. NCS produces a threshold-shaped dose-response, while ETP produces a linear, no threshold response in the tested dose-ranges [5]. NCS causes a short burst of oxidative damage that forms double strand breaks. NCS is destroyed during this process, however, and the resulting double strand breaks are susceptible to normal repair processes. ETP binds topoisomerase II and forms a complex with the protein and DNA, forming a lesion that is poorly repaired. We chose these chemicals because (1) they have different dose-response curves for micronucleus response (threshold vs. linear [5]; and (2) the lesions they induce have different susceptibilities to repair. Using high-content imaging with confocal microscopy, HT1080 cells were exposed to varying doses of NCS and ETP and the DRCs were counted across doses and times. With this technique the number of foci (DNA repair centers) can be counted in each cell (Fig. 9A). NCS-mediated DNA damage was rapidly resolved (measured as foci

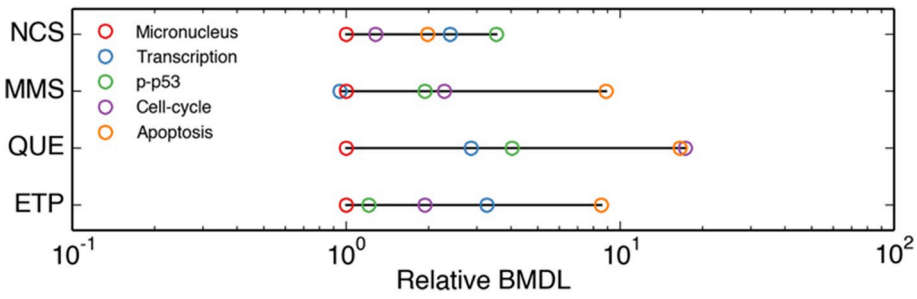

Fig. 7. Relationship between the benchmark doses for several DNA damage endpoints for neocarzinostatin (NCS), methane methylsufonate (MMS), quercetin (QUE), and etoposide (ETP). Because effective concentrations of these compounds vary dramatically, BMDL concentrations (horizontal axis) are expressed relative to the BMD for micronucleus formation. For all four compounds, micronucleus induction (red) is the most sensitive—or nearly so—endpoint. With the exception of MMS, coordinated transcriptional responses do not occur at concentrations that cause micronuclueus formation. Figure reproduced from McMullen et al. [5].

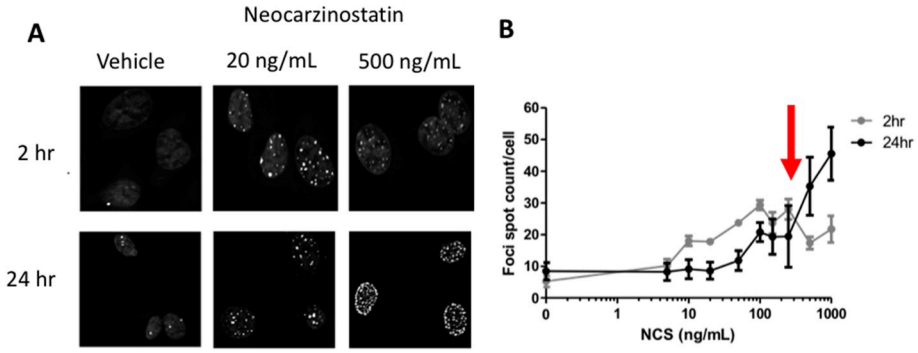

Fig. 9. DNA repair centers in human cells 2 or 24 h after exposure to neocarzinostatin. (A) Representative image panels are shown highlighting pH2AX accumulation (60x magnification). (B) Quanitation of DRCs at 2 and 24 h for various doses of NCS. At low doses, DRCs are formed at 2 h but are no longer present at 24 h. At high doses, more DRCs are present at later time points, indicating accumulated damage and poor repair capacity. Interestingly, in contrast to NCS, low concentrations of ETP shows long-lived DRCs (up to 24 h), which are also associated with micronuclei (red arrow). Thus, when post-translational repair is not efficient, the threshold shaped dose-response is not observed for micronucleus induction. Updated from Ref. [158,159].

dissolution) at low levels of exposure (Fig. 9A, middle panel; Fig. 9B grey line is above black line); at 24 h there were less foci than shortly after exposure, indicating successful repair. However, at high concentrations of NCS, DNA damage is retained at 24 h (Fig. 9A, right panel; Figure B black line is above grey line). This indicates that the post-translational repair processes (DRCs) can prevent long-term damage at low doses. Higher doses that saturate these post-translational processes are the same as those that cause permanent chromosomal changes (i.e., micronuclei induction; [5]. Thus, this transition from post-translational response (DNA repair center formation) to transcriptional response represents a “tipping point” between adaptation and adversity (Fig. 9B, red arrow).

Interestingly, in contrast to NCS, low concentrations of ETP shows long-lived DRCs (up to 24 h), which are also associated with micronuclei (Fig. 10). Thus, when post-translational repair is not efficient, the threshold shaped dose-response is not observed for micronucleus induction. It is possible that a threshold may be observed with lower doses of ETP, as was the case with ethyl nitrosourea in the Doak et al. [144,145] and Johnson et al. [4]; studies. In preliminary studies, removing the ETP after 2 h of treatment and allowing recovery in growth media produces dose-response curves that are more similar to the threshold-shaped dose-resposne curves seen with NCS [159], indicating that repair can prevent permanent damage from ETP when the repair processes are not overwhelmed by ongoing damage. Overall, the studies described here demonstrate that when a threshold is observed in the measure of permanent damage (micronuclei), the tipping point – or threshold dose – is indicative of the concentration at which repair is no longer efficient.

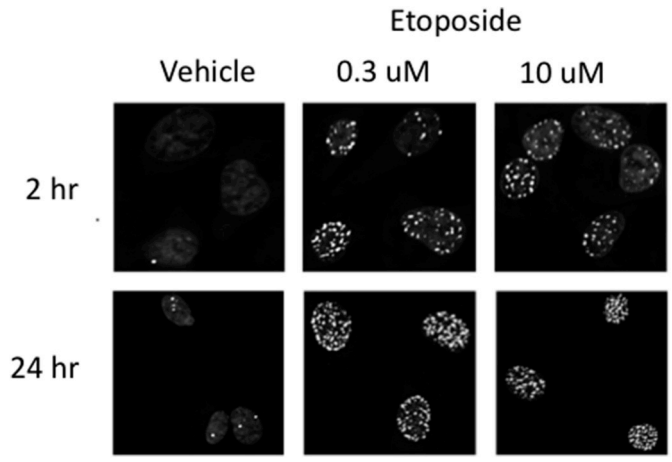

Fig. 10. DNA repair centers in human cells 2 or 24 h after exposure to etoposide. Representative image panels are shown highlighting pH2AX accumulation (60x magnification). At low doses, DRCs are formed at 2 h are still present at 24 h. No resolution of DRCs is observed. Updated from [159].

The ability to measure DNA damage and repair at doses below those inducing micronuclei give us an unprecedented look at the cellular dynamics that define the shape of the micronucleus curve. DNA repair center formation at doses well below those where permanent damage (micronuclei) or transcriptional activation occur make clear that there are regions of dose where DNA damage and DNA repair are active before activation of these other processes. DNA repair center formation (a sentinel of DNA damage) was present at 10-fold lower concentrations than those leading to micronuclei or transcription. More importantly, the resolution of this damage through the post-translational formation of DNA repair centers appears to prevent conversion of transient breaks to permanent damage. These studies indicate that the threshold in micronuclei response to NCS is not due to assay artifact: it is a true biological threshold resulting from repair activity within the cell.

3. Conclusions

As our understanding of biology increases and we gain access to ever-evolving technology, we must continue to apply the best science to the discipline of human health and risk assessment. This involves continually challenging the status quo, default assumptions, and the bias that is inherent in human efforts to assess chemical risk. Here, we provide several examples that demonstrate how increased understanding of the biology of cancer can guide experimental design and how, with targeted experiments, it is possible to identify the key processes that underpin the dose-response for chemical carcinogens. These processes include protective mechanisms at the molecular, cellular and organism level that prevent low level exposures from causing long-term consequences. With the advances in technology that allow us to evaluate these mechanisms at the molecular, cellular and pathway level, we have the ability to move beyond default approaches to biologically based understanding of chemical dose–response.

Declaration of interest

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Appendix A. Supplementary data

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Transparency document

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References

[1] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (5) (2011) 646–674.
[2] S.M. Bryce, J. Shi, J. Nicolette, M. Diehl, P. Sonders, S. Avlasevich, S. Raja, J.C. Bemis, S.D. Dertinger, High content flow cytometric micronucleus scoring method is applicable to attachment cell lines, Environ. Mol. Mutagen. 51 (2010) 260–266.
[3] E. Gocke, L. Müller, In vivo studies in the mouse to define a threshold for the genotoxicity of EMS and ENU, Mutat. Res. 78 (2) (2009) 101–107.
[4] G.E. Johnson, S.H. Doak, S.M. Griffiths, E.L. Quick, D.O. Skibinski, Z.M. Zaïr, G.J. Jenkins, Non-linear dose-response of DNA-reactive genotoxins: recommendations for data analysis, Mutat. Res. 678 (2) (2009) 95–100.
[5] P.D. McMullen, S. Pendse, Y. Adeleye, P.L. Carmichael, M.E. Andersen, R.A. Clewell, Using transcriptomics to evaluate thresholds in genotoxicity dose-response, in: M. Waters, R. Thomas (Eds.), Toxicogenomics in Predictive Carcinogenicity: Current Issues and Future Applications, Royal Society of Canada Press. RSC Publishing, 2016.
[6] L.H. Pottenger, M.R. Schisler, F. Zhang, M.J. Bartels, D.D. Fontaine, L.G. McFadden, Dose-response and operational thresholds/NOAELs for in vitro mutagenic effects from DNA-reactive mutagens, MMS and MNU, Mutat. Res. 678 (2) (2009) 138–147.
[7] W.K. Lutz, R.W. Lutz, Statistical model to estimate a threshold dose and its confidence limits for the analysis of sublinear dose-response relationships, exemplified for mutagenicity data, Mutat. Res. 678 (2) (2009) 118–122.
[8] R.A. Clewell, B. Sun, Y. Adeleye, P. Carmichael, A. Efremenko, P.D. McMullen, S. Pendse, O.J. Trask, A. White, M.E. Andersen, Profiling dose-dependent activation of p53 mediated signaling pathways by chemicals with distinct mechanisms of DNA damage, Toxicol. Sci. 142 (1) (2014) 56–73.
[9] M. Hoshi, K. Morimura, X. Wanibuchi, M. Wei, E. Okochi, T. Ushijima, K. Takaoka, S. Fukushima, No-observed effect levels for carcinogenicity and for in vivo mutagenicity of a genotoxic carcinogen, Toxicol. Sci. 81 (2) (2004) 273–279.
[10] W. Slikker Jr., M.E. Andersen, M.S. Bogdanffy, J.S. Bus, S.D. Cohen, R.B. Conolly, R.M. David, N.G. Doerrer, D.C. Dorman, D.W. Gaylor, D. Hattis, J.M. Rogers, R.W. Setzer, J.A. Swenberg, K. Wallace, Dose-dependent transitions in mechanisms of toxicity: case studies, Toxicol. Appl. Pharmacol. 201 (3) (2004) 226–294.
[11] G.M. Williams, M.J. Iatropoulos, A.M. Jeffrey, Mechanistic basis for nonlinearities and thresholds in rat liver carcinogenesis by the DNA-reactive carcinogens 2-acetylaminofluorene and diethylnitrosamine, Toxicol. Pathol. 28 (3) (2000) 388–395.
[12] J.R. Brown, J.L. Thornton, Percivall Pott (1714-1788) and chimney sweepers' cancer of the scrotum, Br. J. Ind. Med. 14 (1) (1957) 68–70.
[13] O.T. Avery, C.M. MacLeod, M. McCarty, Studies on the chemical nature of the substance inducing transformation of pneumoccal types. Induction of transformaton by a desoxyribonucleic acid fraction isolated from pneumoccus type III, J. Exp. Med. 79 (1944) 137–158.
[14] J.D. Watson, F.H. Crick, Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid, Nature 171 (1953) 737–738.
[15] P.N. Magee, J.M. Barnes, The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine, Br. J. Canc. 10 (1956) 114–122.
[16] M.B. Sporn, C.W. Dingman, H.L. Phelps, G.N. Wogan, Aflatoxin B1: binding to DNA in vitro and alteration of RNA metabolism in vivo, Science 151 (1966) 1539–1541.
[17] C.C. Harris, A.L. Frank, C. van Haaften, D.G. Kaufman, R. Connor, F. Jackson, L.A. Barrett, E.M. McDowell, B.F. Trump, Binding of (3H)benzo(a)pyrene to DNA in cultured human bronchus, Cancer Res. 36 (3) (1976) 1011–1018.
[18] S.M. Friedman, K. Villamil, R.A. Suriano, B.P. Egolf, Alar and apples: newspapers, risk and media responsibility, Publ. Understand. Sci. 5 (1) (1996) 1–20.
[19] K.S. Crump, D.G. Hoel, C.H. Langley, R. Peto, Fundamental carcinogenic processes and their implications for low dose risk assessment, Cancer Res. 36 (9 pt.1) (1976) 2973–2979.
[20] NAS, Risk Assessment in the Federal Government: Managing the Process, The National Academies Press, Washington, DC, 1983.
[21] R.L. Kathren, Pathway to a paradigm: the linear non-threshold dose-response model in historical context. The American Academy of Health Physics. Radiology Centennial Hartman Oration, Health Phys. 70 (5) (1995) 621–635.
[22] R.E. Albert, R.E. Train, E. Anderson, Rationale developed by the Environmental Protection Agency for the assessment of carcinogenic risks, J. Natl. Cancer Inst. 58 (5) (1977) 1537–1541.
[23] USEPA, Guidelines for carcinogen risk assessment. EPA/630/R-00/004, Fed. Regist. 51 (185) (1986) 33992–43003.
[24] K.S. Crump, B.C. Allen, R.B. Howe, P.W. Crockett, Time-related factors in quantitative risk assessment, J. Chron. Dis. 40 (Suppl 2) (1987) 101S–111S.
[25] S.H. Moolgavkar, M. Hakama, R. Peto, R. Doll, The multistage theory of carcinogenesis, Int. J. Cancer 19 (5) (1977) 730–731.
[26] S.H. Moolgavkar, Multistage models for carcinogenesis, J. Natl. Cancer Inst. 65 (2) (1980) 215–216.
[27] S.H. Moolgavkar, Carcinogenesis modeling: from molecular biology to epidemiology, Annu. Rev. Public Health 7 (1986) 151–169.
[28] S.H. Moolgavkar, A.G. Knudson, Mutation and cancer: a model for human carcinogenesis, J. Natl. Cancer Inst. 66 (1981) 1037–1052.
[29] S.H. Moolgavkar, E.G. Luebeck, Incorporating cell proliferation kinetics into models for cancer risk assessment, Toxicology 102 (1–2) (1995) 141–147.
[30] K.T. Bogen, An adjustment factor for mode-of-action uncertainty with dual-mode carcinogens: the case of naphthalene-induced nasal tumors in rats, Risk Anal. 28 (4) (2008) 1033–1051.
[31] C.W. Chen, Biologically based dose-response model for liver tumors induced by trichloroethylene, Environ. Health Perspect. 108 (SUPPL. 2) (2000) 335–342.
[32] K.H. Liao, Y.M. Tan, R.B. Conolly, H.J. Clewell, Bayesian estimation of pharmacokinetic and pharmacodynamic parameters in a mode-of-action based cancer risk assessment for chloroform, Risk Anal. 27 (6) (2007) 1535–1551.
[33] L.M. Sweeney, C.R. Kirman, R.J. Albertini, Y.M. Tan, H.J. Clewell, J.G. Filser, G. Csanády, L.H. Pottenger, M.I. Banton, C.J. Graham, L.S. Andrews, R.J. Papciak, M.L. Gargas, Derivation of inhalation toxicity reference values for propylene oxide using mode of action analysis: example of a threshold carcinogen, Crit. Rev. Toxicol. 39 (6) (2009) 462–486.
[34] S.M. Cohen, L.B. Ellwein, Relationship of DNA adducts derived from 2-acetylaminofluorene to cell proliferation and the induction of rodent liver and bladder tumors, Toxicol. Pathol. 23 (2) (1995) 136–142.
[35] R.B. Conolly, J.S. Kimbell, D. Janszen, P.M. Schlosser, D. Kalisak, J. Preston, et al., Biologically motivated computational modeling of formaldehyde carcinogenicity in the F344 rat, Toxicol. Sci. 75 (2003) 432–447.
[36] R.B. Conolly, J.S. Kimbell, D. Janszen, P.M. Schlosser, D. Kalisak, J. Preston, et al., Human respiratory tract cancer risks of inhaled formaldehyde: dose-response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset, Toxicol. Sci. 82 (2004) 279–296.
[37] J.A. Swenberg, B.C. Moeller, K. Lu, J.E. Rager, R.C. Fry, T.B. Starr, Formaldehyde carcinogenicity research: 30 years and counting for mode of action, epidemiology, and cancer risk assessment, Toxicol. Pathol. 41 (2013) 181–189, https://doi.org/10.1177/0192623312466459.
[38] USEPA, Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum, United States Environmental Protection Agency, Washington DC, 2005 EPA/630/P-03/001F.
[39] M.S. Bogdanffy, G. Daston, E.M. Faustman, C.A. Kimmel, G.L. Kimmel, J. Seed, V. Vu, Harmonization of cancer and noncancer risk assessment: proceedings of a consensus-building workshop, Toxicol. Sci. 61 (1) (2001) 18–31.
[40] USEPA, Toxicological Review of Chloroform in Support of Summary Information on the Integrated Risk Information System (IRIS). EPA/635-R01/001, National Center for Environmental Assessment, USEPA, Washington, DC, 2001.
[41] T.A. Jorgenson, E.F. Meierhenry, C.J. Rushbrook, R.J. Bull, M. Robinson, Carcinogenicity of chloroform in drinking water to male Osborne-Mendel rats and female B6C3F1 mice, Fundam. Appl. Toxicol. 5 (4) (1985) 760–769.
[42] E.J. Calabrese, M.E. McCarthy, E. Kenyon, The occurrence of chemically induced hormesis, Health Phys. 52 (5) (1987) 531–541.
[43] E.J. Calabrese, L.A. Baldwin, Can the concept of hormesis Be generalized to carcinogenesis? Regul. Toxicol. Pharmacol. 28 (3) (1998) 230–241.
[44] M. Cieślik, A.M. Chinnaiyan, Cancer transcriptome profiling at the juncture of clinical translation, Nat. Rev. Genet. 19 (2018) 93–109 2018.
[45] A. Fabregat, S. Jupe, L. Matthews, K. Sidiropoulos, M. Gillespie, P. Garapati, R. Haw, B. Jassal, F. Korninger, B. May, M. Milacic, C.D. Roca, K. Rothfels, C. Sevilla, V. Shamovsky, S. Shorser, T. Varusai, G. Viteri, J. Weiser, G. Wu, L. Stein, H. Hermjakob, P. D'Eustachio, The reactome pathway knowledgebase, Nucleic Acids Res. 46 (D1) (2018) D649–D655.
[46] Gene Ontology Consortium, The Gene Ontology (GO) database and informatics resource, Nucleic Acids Res. 32 (suppl_1) (2004) D258–D261.
[47] M. Kanehisa, S. Goto, KEGG: kyoto encyclopedia of genes and genomes, Nucleic Acids Res. 28 (2000) 27–30.
[48] H.H. Li, D.R. Hyduke, R. Chen, P. Heard, C.L. Yauk, J. Aubrecht, A.J. Fornace Jr., Development of a toxicogenomics signature for genotoxicity using a dose-optimization and informatics strategy in human cells, Environ. Mol. Mutagen. 56 (6) (2015) 505–519.
[49] R.S. Thomas, S.C. Wesselkamper, N.C. Wang, Q.J. Zhao, D.D. Petersen, J.C. Lambert, I. Cote, L. Yang, E. Healy, M.B. Black, H.J. Clewell 3rd, B.C. Allen, M.E. Andersen, Temporal concordance between apical and transcriptional points of departure for chemical risk assessment, Toxicol. Sci. 134 (1) (2013) 180–194.
[50] R.S. Thomas, B.C. Allen, A. Nong, L. Yang, E. Bermudez, H.J. Clewell IIIet al., A method to integrate benchmark dose estimates with genomic data to access the functional effects of chemical exposure, Toxicol. Sci. 98 (2007) 240–248.
[51] R.S. Thomas, H.J. Clewell 3rd, B.C. Allen, S.C. Wesselkamper, N.C. Wang, J.C. Lambert, J.K. Hess-Wilson, Q.J. Zhao, M.E. Andersen, Application of transcriptional benchmark dose values in quantitative cancer and noncancer risk assessment, Toxicol. Sci. 120 (1) (2011) 194–205.
[52] R. Farmahin, A. Williams, B. Kuo, N.L. Chepelev, R.S. Thomas, T.S. Barton-Maclaren, I.H. Curran, A. Nong, M.G. Wade, C.L. Yauk, Recommended approaches in the application of toxicogenomics to derive points of departure for chemical risk assessment, Arch. Toxicol. 91 (5) (2017) 2045–2065.
[53] A.F. Webster, N. Chepelev, R. Gagné, B. Kuo, L. Recio, A. Williams, C.L. Yauk, Impact of genomics platform and statistical filtering on transcriptional benchmark

doses (BMD) and multiple approaches for selection of chemical point of departure (PoD), PLoS One 10 (8) (2015) e0136764.
[54] K. Boehme, Y. Dietz, P. Hewitt, S.O. Mueller, Genomic profiling uncovers a molecular pattern for toxicological characterization of mutagens and promutagens in vitro, Toxicol. Sci. 122 (2011) 185–197.
[55] H. Ellinger-Ziegelbauer, H. Gmuender, A. Bandenburg, H.J. Ahr, Prediction of a carcinogenic potential of rat hepatocarcinogens using toxicogenomics analysis of short-term in vivo studies, Mutat. Res. 637 (2008) 23–39.
[56] M. Iida, C.H. Anna, J. Hartis, M. Bruno, B. Wetmore, J.R. Dubin, S. Sieber, L. Bennett, M.L. Cunningham, R.S. Paules, K.B. Tomer, C.D. Houle, A.B. Merrick, R.C. Sills, T.R. Devereux, Changes in global gene and protein expression during early mouse liver carcinogenesis induced by non-genotoxic model carcinogens oxazepam and Wyeth-14,643, Carcinogenesis 24 (2003) 757–770.34.
[57] ECHA, Opinion on Scientific Evaluation of Occupational Exposure Limits for Nickel and its Compounds. ECHA/RAC/A77-O-0000001412-86-189/F. 9 March 2018, (2018).
[58] A.Y. Efremenko, J.L. Campbell Jr., D.E. Dodd, A.R. Oller, H.J. Clewell 3rd, Time- and concentration-dependent genomic responses of the rat airway to inhaled nickel subsulfide, Toxicol. Appl. Pharmacol. 279 (3) (2014) 441–454.
[59] A.Y. Efremenko, J.L. Campbell, D.E. Dodd, A.R. Oller, H.J. Clewell 3rd, Time- and concentration-dependent genomic responses of the rat airway to inhaled nickel sulfate, Environ. Mol. Mutagen. 58 (8) (2017) 607–618.
[60] G. Patlewicz, C. Kuseva, A. Kesova, I. Popova, T. Zhechev, T. Pavlov, D.W. Roberts, O. Mekenyan, Towards AOP application–implementation of an integrated approach to testing and assessment (IATA) into a pipeline tool for skin sensitization, Regul. Toxicol. Pharmacol. 69 (3) (2014) 529–545.
[61] USEPA, Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing, United States Environmental Protection Agency, Washington DC, 2018 EPA-HQ-OPP-2016-0093-0090.
[62] B.N. Ames, W.E. Durston, E. Yamasaki, F.D. Lee, Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection, Proc. Natl. Acad. Sci. U. S. A 70 (8) (1973) 2281–2285.
[63] A. Ivashkevich, C.E. Redon, A.J. Nakamura, R.F. Martin, O.A. Martin, Use of the γ-H2AX assay to monitor DNA damage and repair in translational cancer research, Cancer Lett. 327 (1–2) (2012) 123–133.
[64] G.E. Johnson, Mammalian cell HPRT gene mutation assay: test methods, Methods Mol. Biol. 817 (2012) 55–67.
[65] L. Luzhna, P. Kathiria, O. Kovalchuk, Micronuclei in genotoxicity assessment: from genetics to epigenetics and beyond, Front. Genet. 4 (2013) 131.
[66] A.K. Olsen, S.D. Dertinger, C.T. Krüger, D.M. Eide, C. Instanes, G. Brunborg, A. Hartwig, A. Graupner, The pig-a gene mutation assay in mice and human cells: a review, Basic Clin. Pharmacol. Toxicol. 121 (Suppl 3) (2017) 78–92.
[67] B.J. Rees, M. Tate, A.M. Lynch, C.A. Thornton, G.J. Jenkins, R.M. Walmsley, G.E. Johnson, Development of an in vitro PIG-A gene mutation assay in human cells, Mutagenesis 32 (2) (2017) 283–297.
[68] D.L. Wyborski, S. Malkhosyan, J. Moores, M. Perucho, J.M. Short, Development of a rat cell line containing stably integrated copies of a lambda/lacI shuttle vector, Mutat. Res. 334 (2) (1995) 161–165.
[69] J.T. MacGregor, R. Frötschl, P.A. White, K.S. Crump, D.A. Eastmond, S. Fukushima, M. Guérard, M. Hayashi, L.G. Soeteman-Hernández, T. Kasamatsu, D.D. Levy, T. Morita, L. Müller, R. Schoeny, M.J. Schuler, V. Thybaud, G.E. Johnson, IWGT report on quantitative approaches to genotoxicity risk assessment I. Methods and metrics for defining exposure-response relationships and points of departure (PoDs), Mutat. Res. Genet. Toxicol. Environ. Mutagen 783 (2015) 55–65.
[70] J.T. MacGregor, R. Frötschl, P.A. White, K.S. Crump, D.A. Eastmond, S. Fukushima, M. Guérard, M. Hayashi, L.G. Soeteman-Hernández, G.E. Johnson, T. Kasamatsu, D.D. Levy, T. Morita, L. Müller, R. Schoeny, M.J. Schuler, V. Thybaud, IWGT report on quantitative approaches to genotoxicity risk assessment II. Use of point-of-departure (PoD) metrics in defining acceptable exposure limits and assessing human risk, Mutat. Res. Genet. Toxicol. Environ. Mutagen 783 (2015) 66–78.
[71] Chemical Industry Institute of Toxicology (CIIT), Formaldehyde: Hazard Characterization and Dose-Response Assessment for Carcinogenicity by the Route of Inhalation, revised edition, (1999).
[72] W.D. Kerns, K.L. Pavkov, D.J. Donofrio, E.J. Gralla, J.A. Swenberg, Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure, Cancer Res. 43 (1983) 4382–4392.
[73] T.M. Monticello, J.A. Swenberg, E.A. Gross, J.R. Leininger, J.S. Kimbell, S. Seikop, et al., Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells, Cancer Res. 56 (1996) 1012–1022.
[74] M. Casanova, K.T. Morgan, E.A. Gross, O.R. Moss, H.D. Heck, DNA-protein cross-links and cell replication at specific sites in the nose of F344 rats exposed subchronically to formaldehyde, Fundam. Appl. Toxicol. 23 (1994) 525–536.
[75] H.D. Heck, M. Casanova, T.B. Starr, Formaldehyde toxicity – new understanding, Crit. Rev. Toxicol. 20 (1990) 397–426.
[76] R.B. Conolly, K.T. Morgan, M.E. Andersen, T.M. Monticello, H.J. Clewell, A biologically-based risk assessment strategy for inhaled formaldehyde, Comments Toxicol. 4 (1992) 269–293.
[77] R.B. Conolly, The use of biologically based modeling in risk assessment, Toxicology 181–182 (2002) 275–279.
[78] J.S. Kimbell, R.P. Subramaniam, Use of computational fluid dynamics models for dosimetry of inhaled gases in the nasal passages, Inhal. Toxicol. 13 (5) (2001) 325–334.
[79] M. Casanova, D.F. Deyo, H.D. Heck, Covalent binding of inhaled formaldehyde to DNA in the nasal mucosa of Fischer 344 rats: analysis of formaldehyde and DNA by high-performance liquid chromatography and provisional pharmacokinetic interpretation, Fundam. Appl. Toxicol. 12 (3) (1989) 397–417.
[80] R.B. Conolly, P.D. Lilly, J.S. Kimbell, Simulation modeling of the tissue disposition of formaldehyde to predict nasal DNA-protein cross-links in Fischer 344 rats, rhesus monkeys, and humans, Environ. Health Perspect. 108 (Suppl 5) (2000) 919–924.
[81] J.S. Kimbell, E.A. Gross, R.B. Richardson, R.B. Conolly, K.T. Morgan, Correlation of regional formaldehyde flux predictions with the distribution of formaldehyde-induced squamous metaplasia in F344 rat nasal passages, Mutat. Res. 380 (1–2) (1997) 143–154.
[82] T.M. Monticello, F.J. Miller, K.T. Morgan, Regional increases in rat nasal epithelial cell proliferation following acute and subchronic inhalation of formaldehyde, Toxicol. Appl. Pharmacol. 111 (1991) 409–421.
[83] M.E. Andersen, H.J. Clewell III, E. Bermudez, G.A. Willson, R.S. Thomas, Genomic signatures and dose-dependent transitions in nasal epithelial responses to inhaled formaldehyde in the rat, Toxicol. Sci. 105 (2008) 368–383.
[84] M.E. Andersen, H.J. Clewell III, E. Bermudez, D.E. Dodd, G.A. Willson, J.L. Campbell, et al., Formaldehyde: integrating dosimetry, cytotoxicity, and genomics to understand dose-dependent transitions for an endogenous compound, Toxicol. Sci. 118 (2010) 716–731, https://doi.org/10.1093/toxsci/kfq303.
[85] ECHA, Formaldehyde Registration Dossier. EC Number 200-001-8 CAS Number 50-00-0. European Chemicals Agency, (2011) Available at: https://echa.europa.eu/registration-dossier/-/registered-dossier/15858.
[86] SCOEL, SCOEL/REC/125 Formaldehyde Recommendation from the Scientific Committee on Occupational Exposure Limits (SCOEL) of the Euproean Commission. Scientific Committee on Occupational Exposure Limits: Bolt HM, Johanson G, Nielsen GD, Papameletiou D, Klein CL, (2016) Available at: https://publications.europa.eu/en/publication-detail/-/publication/7a7ae0c9-c03d-11e6-a6db-01aa75ed71a1/language-en.
[87] USEPA, Toxicological review of formaldehyde – inhalation assessment (CAS No. 50-00-0), Support of Summary Information on the Integrated Risk Information System (IRIS). Volume I of IV. Introduction, Background, and Toxicokinetics, United States Environmental Protection Agency, Washington DC, 2010EPA/635/R-10/002A.
[88] NRC, Review of Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde. Committee to Review EPA's Draft IRIS Assessment of Formaldehyde, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies, National Research Council of the National Academies, The National Academies Press, Washington, DC, 2011.
[89] Office of Management and Budget (OMB), Updated Principles for Risk Analysis, (2007) Available at: https://www.whitehouse.gov/sites/whitehouse.gov/files/omb/memoranda/2007/m07-24.pdf.
[90] H.J. Clewell, H.J. Andersen, B.J. Blaauboer, On the incorporation of chemical-specific information in risk assessment, Toxicol. Lett. 180 (2008) 100–109.
[91] USEPA, Toxicological Review of Hexavalent Chromium in Support of Summary Information on the Integrated Risk Information System (IRIS), U.S. Environmental Protection Agency, Washinton, D.C, 1998.
[92] I. Moffat, N. Martinova, C. Seidel, C.M. Thompson, Hexavalent chromium in drinking water, J. AWWA (Am. Water Works Assoc.) 110 (2018) E22–E35.
[94] USEPA, Data Summary of the Third Unregulated Contaminant Monitoring Rule (UCMR3), United States Environmental Protection Agency, Washington DC, 2017 EPA 815-S-17-001.
[95] NTP, National Toxicology Program Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate (CAS No. 7789-12-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies), NTP TR 546, NIH Publication No. 08-5887, 2008.
[96] Health Canada, H. E. a. C. S. B. Water, Air Quality Bureau (Eds.), Guidelines for Canadian Drinking Water Quality: Guideline Technical Document — Chromium, Health Canada, Ottawa, Ontario, 2016.
[97] OEHHA, Final Technical Support Document on Public Health Goal for Hexavalent Chromium in Drinking Water. Pesticide and Environmental Toxicology Branch, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, 2011.
[98] A.H. Stern, A quantitative assessment of the carcinogenicity of hexavalent chromium by the oral route and its relevance to human exposure, Environ. Res. 110 (2010) 98–807.
[99] TCEQ, Hexavalent Chromium Oral Reference Dose: Development Support Document (Final), (2016).
[100] C.M. Thompson, C.R. Kirman, S.M. Hays, M. Suh, S.E. Harvey, D.M. Proctor, J.E. Rager, L.C. Haws, M.A. Harris, Integration of mechanistic and pharmacokinetic information to derive oral reference dose and margin-of-exposure values for hexavalent chromium, J. Appl. Toxicol. 38 (2018) 351–365.
[101] USEPA, Toxicological Review of Hexavalent Chromium in Support of Summary Information on the Integrated Risk Information System (IRIS). External Review DRAFT, U.S. Environmental Protection Agency, Washington, D.C, 2010.
[102] C.J. Fahrni, Biological applications of X-ray fluorescence microscopy: exploring the subcellular topography and speciation of transition metals, Curr. Opin. Chem. Biol. 11 (2007) 121–127.
[103] G. Robison, T. Zakharova, S. Fu, W. Jiang, R. Fulper, R. Barrea, M.A. Marcus, W. Zheng, Y. Pushkar, X-ray fluorescence imaging: a new tool for studying manganese neurotoxicity, PLoS One 7 (2012) e48899.
[104] C.M. Thompson, J. Seiter, M.A. Chappell, R.V. Tappero, D.M. Proctor, M. Suh, J.C. Wolf, L.C. Haws, R. Vitale, L. Mittal, C.R. Kirman, S.M. Hays, M.A. Harris, Synchrotron-based imaging of chromium and gamma-H2AX immunostaining in the duodenum following repeated exposure to Cr(VI) in drinking water, Toxicol.

Sci. 143 (2015) 16–25.
[105] M.D. Neal, W.M. Richardson, C.P. Sodhi, A. Russo, D.J. Hackam, Intestinal stem cells and their roles during mucosal injury and repair, J. Surg. Res. 167 (2011) 1–8.
[106] C.S. Potten, C. Booth, D.M. Pritchard, The intestinal epithelial stem cell: the mucosal governor, Int. J. Exp. Pathol. 78 (1997) 219–243.
[107] P. Greaves, Histopathology of Preclinical Toxicity Studies, fourth ed., Elsevier-Academic Press, London, 2012.
[108] N. Barker, R.A. Ridgway, J.H. van Es, M. van de Wetering, H. Begthel, M. van den Born, E. Danenberg, A.R. Clarke, O.J. Sansom, H. Clevers, Crypt stem cells as the cells-of-origin of intestinal cancer, Nature 457 (2009) 608–611.
[109] T.J. O'Brien, H. Ding, M. Suh, C.M. Thompson, B.L. Parsons, M.A. Harris, W.A. Winkelman, J.C. Wolf, J.G. Hixon, A.M. Schwartz, M.B. Myers, L.C. Haws, D.M. Proctor, Assessment of K-Ras mutant frequency and micronucleus incidence in the mouse duodenum following 90-days of exposure to Cr(VI) in drinking water, Mutat. Res. 754 (2013) 15–21.
[110] C.M. Thompson, J.C. Wolf, R.H. Elbekai, M.G. Paranjpe, J.M. Seiter, M.A. Chappell, R.V. Tappero, M. Suh, D.M. Proctor, A. Bichteler, L.C. Haws, M.A. Harris, Duodenal crypt health following exposure to Cr(VI): micronucleus scoring, gamma-H2AX immunostaining, and synchrotron X-ray fluorescence microscopy. Mutation research, Genetic toxicology and environmental mutagenesis 789–790 (2015) 61–66.
[111] C.M. Thompson, D.M. Proctor, M. Suh, L.C. Haws, C.D. Hebert, J.F. Mann, H.G. Shertzer, J.G. Hixon, M.A. Harris, Comparison of the effects of hexavalent chromium in the alimentary canal of F344 rats and B6C3F1 mice following exposure in drinking water: implications for carcinogenic modes of action, Toxicol. Sci. 125 (2012) 79–90.
[112] C.M. Thompson, J.E. Rager, M. Suh, C.L. Ring, D.M. Proctor, L.C. Haws, R.C. Fry, M.A. Harris, Transcriptomic responses in the oral cavity of F344 rats and B6C3F1 mice following exposure to Cr(VI): implications for risk assessment, Environ. Mol. Mutagen. 57 (2016) 706–716.
[113] I.B. Lambert, T.M. Singer, S.E. Boucher, G.R. Douglas, Detailed review of transgenic rodent mutation assays, Mutat. Res. 590 (2005) 1–280.
[114] R.R. Young, C.M. Thompson, H.R. Dinesdurage, R.H. Elbekai, M. Suh, A.C. Rohr, D.M. Proctor, A robust method for assessing chemically induced mutagenic effects in the oral cavity of transgenic Big Blue(R) rats, Environ. Mol. Mutagen. 56 (2015) 629–636.
[115] OECD, Guideline for Testing of Chemicals. 2013. Test Guideline 488, - Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays, adopted July 28 OECD, Paris, France, 2013.
[116] C.M. Thompson, R.R. Young, M. Suh, H.R. Dinesdurage, R.H. Elbekai, M.A. Harris, A.C. Rohr, D.M. Proctor, Assessment of the mutagenic potential of Cr(VI) in the oral mucosa of Big Blue((R)) transgenic F344 rats, Environ. Mol. Mutagen. 56 (2015) 621–628.
[117] C.M. Thompson, R.R. Young, H. Dinesdurage, M. Suh, M.A. Harris, A.C. Rohr, D.M. Proctor, Assessment of the mutagenic potential of hexavalent chromium in the duodenum of big blue(R) rats, Toxicol. Appl. Pharmacol. 330 (2017) 48–52.
[118] C.M. Thompson, D.M. Proctor, M. Suh, L.C. Haws, C.R. Kirman, M.A. Harris, Assessment of the mode of action underlying development of rodent small intestinal tumors following oral exposure to hexavalent chromium and relevance to humans, Crit. Rev. Toxicol. 43 (2013) 244–274.
[119] S.M. Cohen, L.B. Ellwein, Cell proliferation in carcinogenesis, Science 249 (1990) 1007–1011.
[120] C. Tomasetti, B. Vogelstein, Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions, Science 347 (2015) 78–81.
[121] R.A. Brooks, N.J. Gooderham, R.J. Edwards, A.R. Boobis, D.J. Winton, The mutagenicity of benzo[a]pyrene in mouse small intestine, Carcinogenesis 20 (1999) 109–114.
[122] ERG, Peer Review Workshop for EPA's Draft Toxicological Review of Hexavalent Chromium, Eastern Research Group, 2011, http://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=502646.
[123] J. Haney Jr., Consideration of non-linear, non-threshold and threshold approaches for assessing the carcinogenicity of oral exposure to hexavalent chromium, Regul. Toxicol. Pharmacol. 73 (2015) 34–52.
[124] ATSDR, Toxicological Profile for Chlorinated Dibenzo-P-Dioxins, Agency for Toxic Substances and Disease Registry, Atlanta, GA, 1998.
[125] NTP, Report on Carcinogens, Addendum to the Ninth Edition, U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Washinton, D.C, 2001.
[126] T. Simon, L.L. Aylward, C.R. Kirman, J.C. Rowlands, R.A. Budinsky, Estimates of cancer potency of 2,3,7,8-tetrachlorodibenzo(p)dioxin using linear and nonlinear dose-response modeling and toxicokinetics, Toxicol. Sci. 112 (2) (2009) 490–506.
[127] K. Steenland, P. Bertazzi, A. Baccarelli, M. Kogevinas, Dioxin revisited: developments since the 1997 IARC classification of dioxin as a human carcinogen, Environ. Health Perspect. 112 (13) (2004) 1265–1268.
[128] NTP, Carcinogenesis Bioassay of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin in Osborne-Mendel Rats and B6C3F1 Mice, U.S. Department of Health and Human Services National Toxicology Program, Washington, D.C., 1982.
[129] NTP, Toxicology and Carcinogenesis Studies of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD), U.S. Department of Health and Human Services National Toxicology Program, Washington, DC, 2006.
[130] J.E. Gielen, F.M. Goujon, D.W. Nebert, Genetic regulation of aryl hydrocarbon hydroxylase induction. II. Simple Mendelian expression in mouse tissues in vivo, J. Biol. Chem. 247 (4) (1972) 1125–1137.
[131] G.W. Lucier, C.J. Portier, M.A. Gallo, Receptor mechanisms and dose-response models for the effects of dioxins, Environ. Health Perspect. 101 (1993) 36–44.
[132] J.J. Mills, M.E. Andersen, Dioxin hepatic carcinogenesis: biologically motivated modeling and risk assessment, Toxicol. Lett. 68 (1993) 177–189.
[133] A. Poland, E. Glover, 2,3,7,8,-Tetrachlorodibenzo-p-dioxin: segregation of toxocity with the Ah locus, Mol. Pharmacol. 17 (1) (1980) 86–94.
[134] A. Poland, E. Glover, A.S. Kende, Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. Evidence that the binding species is receptor for induction of aryl hydrocarbon hydroxylase, J. Biol. Chem. 251 (16) (1976) 4936–4946.
[135] S. Safe, Molecular biology of the Ah receptor and its role in carcinogenesis, Toxicol. Lett. 120 (2001) 1–7.
[136] USEPA, Health Assessment Document for Polychlorinated Dibenzo-P-Dioxins, United States Environmental Protection Agency, Washington DC, 1985 EPA/600/8-84/014F (NTIS PB86122546).
[137] USEPA, Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (Tcdd) and Related Compounds: Science Advisory Board (External Review Draft) (2000) (Report), (2000).
[138] NAS, Toxicity Testing in the 21st Century: A Vision and a Strategy, The National Academies Press, Washington, DC, 2007.
[139] H.C. Pitot, T.L. Goldsworthy, S. Moran, W. Kennan, H.P. Glauert, R.R. Maronpot, H.A. Campbell, A method to quantitate the relative initiating and promoting potencies of hepatocarcinogenic agents in their dose-response relationships to altered hepatic foci, Carcinogenesis 8 (10) (1987) 1491–1499.
[140] USEPA, EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments, United States Evironmental Protection Agency, Washington DC, 2010 EPA/600/R-10/038A.
[141] NRC, Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment, National Academies Press, Washington, D.C, 2006.
[142] S.A.B. USEPA, SAB Review of EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments (May 2010); EPA-SAB-011-014, (2011).
[143] USEPA, Integrated Risk Information System Chemical Assessment Summary. 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD); CASRN 1746-01-6, (2012).
[144] S.H. Doak, K. Brüsehafer, E. Dudley, E. Quick, G. Johnson, R.P. Newton, G.J. Jenkins, No-observed effect levels are associated with up-regulation of MGMT following MMS exposure, Mutat. Res. 15:648 (1–2) (2008) 9–14.
[145] S.H. Doak, G.J. Jenkins, G.E. Johnson, E. Quick, E.M. Parry, J.M. Parry, Mechanistic influences for mutation induction curves after exposure to DNA-reactive carcinogens, Cancer Res. 67 (8) (2007) 3904–3911.
[146] B. Sun, S.M. Ross, S. Rowley, Y. Adeleye, R.A. Clewell, Contribution of ATM and ATR kinase pathways to p53-mediated response in etoposide and methyl methanesulfonate induced DNA damage, Environ. Mol. Mutagen. 58 (2) (2017) 72–83.
[147] B. Sun, S.M. Ross, O.J. Trask, P. Carmichael, A. White, M. Dent, M.E. Andersen, R.A. Clewell, Assessing dose-dependent differences in DNA-damage, p53 response and genotoxicity for quercetin and curcumin, Toxicol. Vitro 27 (2013) 1877–1887.
[148] C. Arias-Lopez, I. Lazaro-Trueba, P. Kerr, C.J. Lord, T. Dexter, M. Iravani, A. Ashworth, A. Silva, p53 modulates homologous recombination by transcriptional regulation of the RAD51 gene, EMBO Rep. 7 (2) (2006) 219–224.
[149] Y. Barak, T. Juven, R. Haffner, M. Oren, Mdm2 expression is induced by wild type p53 activity, EMBO J. 12 (1993) 461–468.
[150] J. Chen, I. Sadowski, Identification of the mismatch repair genes PMS2 and MLH1 as p53 target genes by using serial analysis of binding elements, Proc. Natl. Acad. Sci. U. S. A. 102 (13) (2005) 4813–4818.
[151] W.S. El-Deiry, T. Tokino, V.E. Velculescu, D.B. Levy, R. Parsons, J.M. Trent, D. Lin, W.E. Mercer, K.W. Kinzler, B. Vogelstein, WAF1, a potential mediator of p53 tumor suppression, Cell 75 (1993) 817–825.
[152] J.W. Harper, G.R. Adami, N. Wei, K. Keyomarsi, S.J. Elledge, The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases, Cell 75 (1993) 805–816.
[153] T. Miyashita, J.C. Reed, Tumor suppressor p53 is a direct transcriptional activator of the human bax gene, Cell 80 (1995) 293–299.
[154] K. Oda, H. Arakawa, p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53, Cell 102 (2000) 849–862.
[155] E.C. Thornborrow, S. Patel, A.E. Mastropietro, E.E. Schwartzfarb, J.J. Manfredi, A conserved intronic response element mediates direct p53-dependent transcriptional activation of both the human and murine bax genes, Oncogene 21 (2002) 990–999.
[156] Y. Adeleye, M.E. Andersen, R.A. Clewell, et al., Implementing toxicity testing in the 21st century (TT21C): making safety decisions using toxicity pathways, and progress in a prototype risk assessment, Toxicology 332 (2015) 102–111.
[157] Q. Zhang, S. Bhattacharya, J. Pi, R.A. Clewell, P.L. Carmichael, M.E. Andersen, Adaptive posttranslational control in cellular stress response pathways and its relationship to toxicity testing and safety assessment, Toxicol. Sci. 147 (2) (2015) 302–316.
[158] R.A. Clewell, P.D. McMullen, Y. Adeleye, M.E. Andersen, Pathway based toxicology and fit-for-purpose assays, in: C. Eskes, M. Whelan (Eds.), Validating Alternative Methods for Toxicity Testing, Springer International Publishing AG, 2015.
[159] M. Yoon, R.A. Clewell, Moving beyond prioritization towards true in vitro-based safety assessment, The Toxicologist, Supplement to Toxicological Sciences 150 (1) (2016) Abstract #1711.
[163] S.H. Moolgavkar, N.E. Day, R.G. Stevens, Two-stage model for carcinogenesis: Epidemiology of breast cancer in females, J Natl Cancer Inst 65 (3) (1980) 559–569.
[164] TCEQ, Formaldehyde: Development Support Document, Texas Commission on Environmental Quality (2015).
[165] S.T. Al Rashid, G. Dellaire, A. Cuddihy, F. Jalali, M. Vaid, et al., Evidence for the

direct binding of phosphorylated p53 to sites of DNA breaks in vivo. Cancer Research 65 (23) (2005) 10810–10821.

[166] Q. Zhang, S. Bhattacharya, R.B. Connolly, H.J. Clewell, N.E. Kaminski, M.E. Andersen, Molecular signaling network motifs provide a mechanistic basis for cellular threshold responses, Environ Health Perspect 122 (12) (2014) 1261–1270.

[167] G. Brorby, C. Ring, F. Loko, M. Harris, Characterization of hexavalent chromium and total chromium in drinking water. (Poster), Environmental Toxicology and Chemistry North America 39th Annual Meeting November 4-8, 2018. Sacramento, California (2018).

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Ionizing radiations epidemiology does not support the LNT model

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ABSTRACT

Most cancers are multifactorial diseases. Yet, epidemiological modeling of the effect of ionizing radiation (IR) exposures based on the linear no-threshold model at low doses (*LNT*) has generally not included co-exposure to chemicals, dietary, socio-economic and other risk factors also known to cause the cancers imputed to IR. When so, increased cancer incidences are incorrectly predicted by being solely associated with IR exposures. Moreover, to justify application of the *LNT* to low doses, high dose-response data, e.g., from the bombing of Hiroshima and Nagasaki, are linearly interpolated to background incidence (which usually has large uncertainty). In order for this interpolation to be correct, it would imply that the biological mechanisms leading to cancer and those that prevent cancer at high doses are exactly the same as at low doses. We show that linear interpolations are incorrect because both the biological and epidemiological evidence for thresholds, or other non-linearities, are more than substantial. We discuss why the *LNT* model suffers from misspecification errors, multiple testing, and other biases. Moreover, its use by regulatory agencies conflates vague assertions of scientific causation, by conjecturing the LNT, for administrative ease of use.

1. Introduction

According to the linear no-threshold dose-response model (*LNT*) any exposure, other than zero, increases the probability of cancer. This view of ionizing radiation (IR) and chemicals has justified stringent regulations and generated a policy controversy about the evidence of causation for the LNT.

We focus on the strength and realism of the epidemiological evidence currently used, which is mostly observational, and is asserted to support the *LNT*. We show why we disagree and suggest that the *LNT* is a scientific conjecture[1]: it cannot be demonstrated that infinitesimally small doses of IR cause infinitesimally small increases in human cancer mortality over background. Yet, the epidemiological *LNT* (BEIR VII Phase 2, 2006, p. 138) is widely accepted and used for regulatory purposes by public agencies, and is advocated by committees formed by learned societies [1].[2] To wit:

A model that plays a prominent role in radiation epidemiology studies is one in which the RR is a linear function of dose. In its simplest form, $RR(D) = 1 + \beta D$, where D is dose, RR(D) is the relative risk at dose D, and β is the ERR per unit of dose, …. This linear RR model has been used extensively in radiation epidemiology, including studies of A-bomb survivors … persons exposed for medical reasons … and nuclear workers …. The model has served as the basis of cancer risk estimation by three BEIR committees [46–48], by the 2000 UNSCEAR committee (2000b), and by the National Institutes of Health [49]. It also plays an important role in developing the BEIR VII committee's cancer risk estimates ….

BEIR VII Phase 2 (2006, p. 246–247, citations omitted) justifies linearity (i.e., linear or linear-quadratic *LNT* specifications) stating that *'historically, and with the exception of leukemia, there has been little statistical evidence of a need for curvature in the LSS (epidemiological) dose-response models … There is stronger evidence of curvature from radiobiological considerations and experimental results.'* Curvature can easily result in quasi-linearity at low doses, virtual thresholds, as may occur when fitting a purely quadratic function to epidemiological data. The French Academies (2005 [2]; found that radiobiological evidence does

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[1] A *conjecture* is a conclusion based on incomplete information for which there is yet no proof. It is neither a presumption (rebuttable by new evidence) nor a verifiable assumption (because there is insufficient evidence).

[2] Cancer is a multifactorial disease generated through a complicated process of genetic and cellular changes due to background, environmental, and occupational, and other exposures. The excess relative risk for a given type of cancer from those exposures, ERR = (RR – 1), is calculated by adjusting it as: $(R_e - R_n)/(R_n < d >)$; average weighted dose, given by $< d >$, when the absorbed dose is weighted by the type of IR, has units of Sv, (1 Sv = 100 rem). Specifically, *'ERR for a specific disease is the rate of that disease in an exposed population divided by the rate of that disease in the unexposed population minus 1'* (BEIR VII Phase 2, p. 143).

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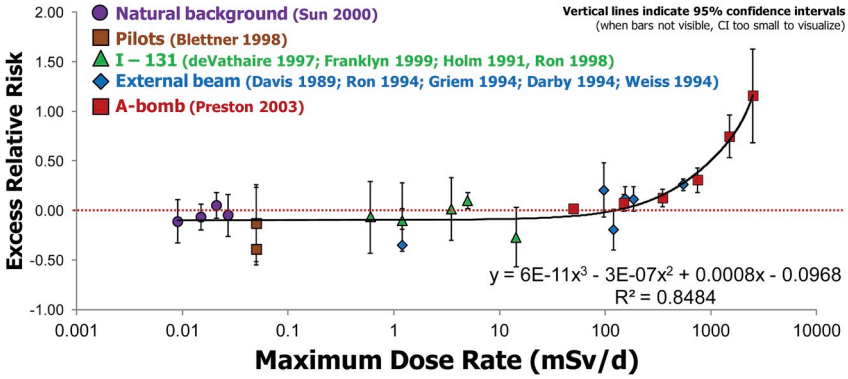

Fig. 1. ERR versus maximum dose rate (mSv/day) from selected epidemiological studies and best-fit model (from the literature in Ref. [7]; Supplemental Table 1). The black line is the third-degree polynomial fit to all data. Dose rates represent whole body effective dose. Error bars are 95% confidence intervals. When error bars are not visible, confidence intervals are indistinguishable from the point. The red dotted line indicates ERR = 0; the ERR is > 0 when maximum dose rate is > 115.85 mSv/d. (F-value (3 df) = 35.418, p-val = 0.000).

not support the *LNT*. We address the basis of this controversy from an epidemiological perspective by:

1) Discussing modeling exposure-response accounting for those exposures and risk factors that are known to cause cancer-specific increased risk.
2) Assessing how to establish the correct functional form (*specification*) of the LNT exposure-response models.
3) Improving the regulatory use of dose-response models by public agencies.

2. Epidemiological results and implications for the LNT

BEIR VII (2006) risk models were largely based on A-bomb survivor data and when risk transportation from a Japanese population to a U.S. population occurred, weighted multiplicative mixes (combinations) of absolute and relative risk models were used for most cancer types. More specifically, although the models used to "transport" risks from the Japanese population to the US population in BEIR VII are more complex, we simplify the discussion by using the LNT relative risk (RR) model extensively utilized in BEIR VII. Here, $RR(D) = 1 + \beta D$, is theoretically and empirically questionable because, at low doses, thresholds and biphasic responses occur [3–5], and because this model is incorrectly specified. Other formulations of the LNT often use the excess relative risk (ERR), but the same issues affect them. An ERR = 0 is no effect; a lower ERR value than 0 is a possibly positive effect, and a larger ERR value an adverse effect. We followed the recent literature, studied the data reported in it, and developed several aggregate empirical relationships between exposure (e.g., mSV/day, mGy, Bq/m^3) and ERR. We accounted for statistical uncertainty by assessing the statistical significance of the ERR and not just its magnitude. Our analyses demonstrated that most of the ERRs (conditional on low doses or exposures) are statistically insignificant (the 95% or 90% confidence intervals straddle ERR = 0.00), in agreement with the null hypothesis of no effect from exposure to low IR doses.

To exemplify model specification error (a critical flaw of the LNT at low doses – discussed later in more detail), suppose that a researcher wishes to describe the association between human heights and weights. A naive epidemiological model might be linear: Height = f (Weight) = a + b(Weight), which is descriptive, but clearly insufficient for prediction.[3] It cannot answer the research question: is weight a predictor of height? The statistical form of the model would be: H = a + bW + e, where *e* is the random errors (often assumed to be normally distributed with mean zero and variance that equals 1). Hence *H* is a random variable, but *W* is not: *W* is deterministic (an assumption that can be relaxed, as discussed later in this paper). The predictive aspect of this simple model can be improved by accounting for age, race, physical activity, diet, etc. The correctly specified model should also be based on the physics of the skeleton, muscle and adipose tissue mass, and include physical constraint such as height less than 3.0 m and weight less than 500 kg. Descriptively, the simple linear model may be sufficient; predictively, this is not the case. It is this latter situation that matters in the discussions that follow.

To be consistent with the time frame of BEIR VII (2006) and the French Academies (2005) [2], we begin with the data represented by Ref. [7] which analyzed 13 independent epidemiological studies of the ERR of solid cancers from maximum IR dose rate (in mSV/day). We replicate their results and add a best fitting model finding that the null hypothesis of no effect cannot be rejected (no LNT behavior) for exposures from 0 up to > 116 mSv/d (Fig. 1). We are aware that weighted doses in mSv (and related dose rates) are linked to the LNT hypothesis (weights are based in part on LNT lines slopes). However, at present appropriate weights (for different radiation types) for use with threshold and hormetic dose-response relationships have not been established for cancer.

The polynomial in Fig. 1 depicts the average behavior of the ERRs as dose rate (mSv/day) increases, confirming [7] (Fig. 2; Supplemental Table 1). Importantly, the ERRs at dose rates between 0.01 and 100 mSv/day are either beneficial (ERR < 0) or suggest a threshold (ERR = 0). Specifically, four out of five epidemiological results for I-131 radioisotope intake are insignificant; 4 out of 6 studies of external beam radiotherapy are statistically insignificant; the four natural background studies are insignificant as are the two airline pilot values (Supplemental Table 1). Descriptively and in the aggregate, behaviors greater than approximately 10 mSv/d are non-linear and statistically significant; behaviors from < 0.01 mSv to approximately 10 mSv/d are threshold-like. Further descriptive understanding of the aggregate effect of IR at very low doses is depicted in Fig. 2, which depicts a plot of ERR/Gy versus mean cumulative dose (mGy) obtained from several epidemiological occupational and other studies (Supplemental Table 2). Again, the relationship is threshold-like up to more than 500 mGy, with most results being statistically insignificant.

We also analyzed epidemiological studies of residential exposure to radon (an alpha particle emitter, exposure in Becquerel's/cubic meters of air) and lung cancer by comparing the ERR (100 Bq/m^3)$^{-1}$ and mean radon levels (Fig. 3; Supplemental Table 3). The linear relationship is statistically insignificant in 15 out of 17 studies for exposure up to approximately 200 Bq/m^3. Despite this, the US EPA remediation limit for radon levels in homes is currently set to 148 Bq/m^3.

For leukemia [8], evaluates ERR as a function of exposure-group-related mean absorbed radiation dose to red bone marrow, expressed in mGy. These results are statistically insignificant from zero up to approximately 140 mGy. Also, no consideration was given to variability in the baseline risk estimate for unexposed individuals so that confidence intervals near the origin (zero dose group) are under reported. Finally,

[3] Given a sample, it is a simple matter to generate an almost perfect description: a polynomial of sufficiently high degree fits most of the data. But its predictions (essential for public health policy) are certainly questionable, as the bias-variance tradeoff literature demonstrates.

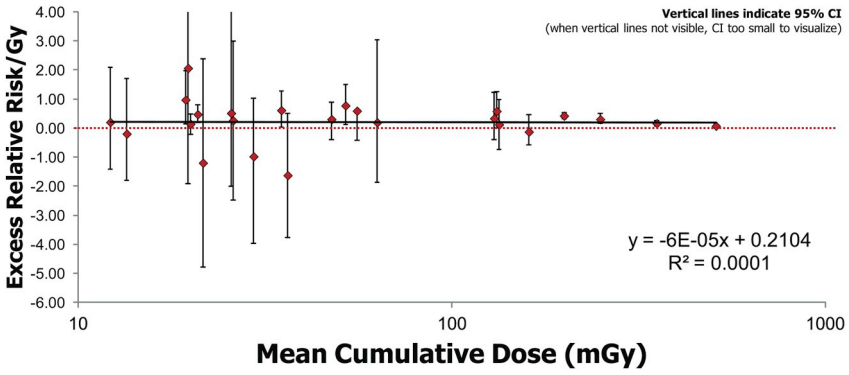

Fig. 2. Summary of epidemiological radiation cancer risk studies represented as ERR/Gy as a function of mean cumulative dose (mGy). The black line is the best linear fit for all data points. Dose rates represent whole body effective dose. Error bars are 95% confidence intervals. When error bars are not visible, confidence intervals are too small relative to the size of the point. The red dotted line is ERR = 0. (F-value = 0.02, 1 df, p-val = 0.963).

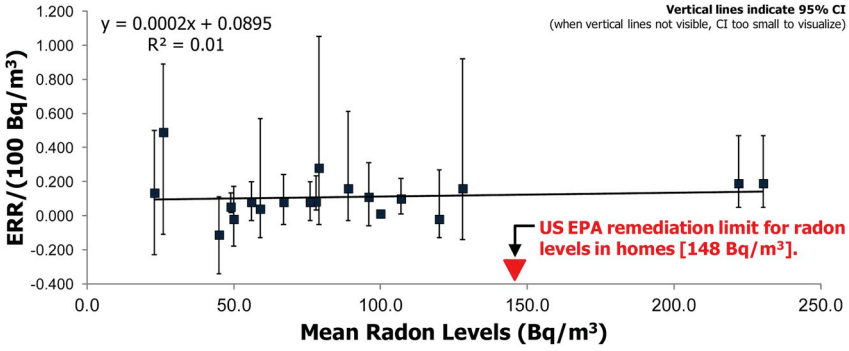

Fig. 3. Lung cancer excess relative risk (ERR) in residential homes due to radon exposure. Summary of epidemiological data on residential radon exposure and lung cancer incidence represented as ERR per 100 Bq/m^3 as a function of mean cumulative radon dose (Bq/m^3). The US EPA standard for radon exposure is set at 148 Bq/m^3. The black line is the best linear fit to the data. Error bars are 95% confidence intervals. When error bars are not visible, the confidence intervals are too small relative to the size of the point. Red dotted line is ERR (100 Bq/m^3) = 0. (F-val = 0.172, df = 1, p-val = 0.684).

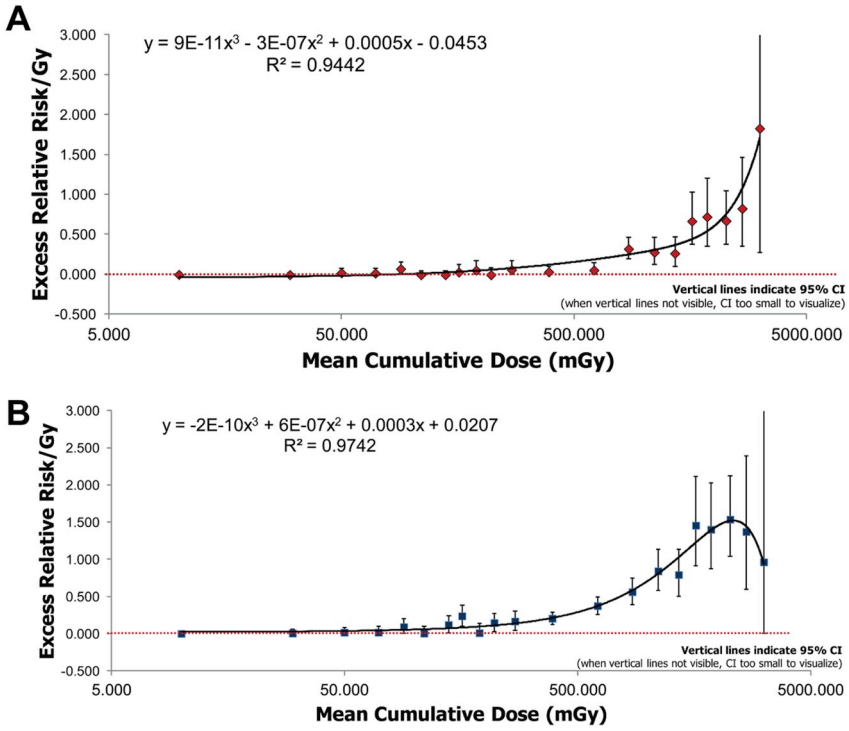

Fig. 4. Summary of solid cancer risk in A-bomb radiation exposed "life-span study" (LSS) in male (A) and female (B) cohorts (adapted from Ref. [9]. Data are ERR/Gy as a function of mean cumulative dose (mGy). The black line is a third-degree polynomial fit to all data points. When error bars are not visible, confidence intervals are too small relative to the point. Red dotted ERR/Gy = 0. For male data (A), F-val = 95.878, df = 3 and p-val = 0.000. For female data (B), F-val = 214.210, df = 3 and p-val = 0.000. Dose errors and possible missing dose from fallout radionuclides could not be addressed in our analyses because of insufficient information.

the Life Span Study (LSS) also shows that results of exposure to IR have a pattern that is consistent with the results discussed: the increased risk (ERR/Gy) begins at exposures that are much greater than 0.1 Gy (100 mGy) (Fig. 4; Supplemental Tables 4 and 5) (Grant et al., 2017).

By way of comparison [10], summarize some of the main IR-related epidemiological studies. For solid cancers, they report the ERRs for the LSS (Hiroshima and Nagasaki), INWORKS (US, UK, France workers), Chernobyl (workers), and results for natural background radiation exposure of the residents of the Techa river basin, Kerala (India), and Yangjiang (China). These results are statistically insignificant at doses from less than 0.1 Sv or Gy to 0.5 Sv or Gy. For leukemia, the LSS, Mayak, and Techa river studies do not report confidence intervals. The results for which the confidence intervals are shown (at doses less than 0.1–0.5 Sv or Gy), are often statistically insignificant (90% or 95% confidence levels). Furthermore [10], assess lung cancer associated with radon exposure (measured in working levels-month (WLM)) from

zero to approximately < 250 WLM. About one-half of the epidemiological results are statistically insignificant (Canada (Newfoundland), Germany) while others are significant (Czech Republic, Germany). Table 1 summarizes modeling issues associated with recent and well-known epidemiological studies.

Most of these issues (none of the epidemiological studies in Table 1 were known to BEIR VII, published in 2006) contradict the earlier statement (BEIR VII Phase 2, p. 246) that '*the committee judges that the balance of scientific evidence at low doses tends to weigh in favor of a simple proportionate relationship between radiation dose and cancer risk.*

2.1. Whither LNT at low doses?

The French Academies (2005) also dealt with the appropriateness of the *LNT*. To wit, the French Academies state that the *LNT* does not account for (parenthetic comments added):

> … all the other potential risk factors (such as tobacco smoking, behavioral, and many other) …. If such factors are present, they must be taken into account by appropriate statistical methods. This point is particularly important with regard to the study of low doses, because the specific effect of the confounding factors can be much greater than the effect of radiation. It is not enough to postulate (in our words, to conjecture) that such a correlation has no logical reason to exist; it is necessary to establish that it did not appear by chance …. For example, in a study investigating the risk of lung cancer due to radon in homes, not taking smoking into account would make the results impossible to interpret ….

The key difficulty is the *misspecification* of the epidemiological *LNT*. To further understand the specification error, the mathematical form of the *LNT* based on ERR, IR dose, *d*, and age at exposure, *e* (BEIR VII Phase 2, p. 143; equation 6–4) is:

$$ERR = \rho(d)_{\beta s}Exp(\gamma e) \quad (1)$$

$\rho(d_{)\beta s}$ is either linear or linear-quadratic, β_s is ERR/Sv, γ is the coefficient for age at exposure. A statistical error term should be added, or otherwise included, when dealing with estimation (e.g., using the Poisson regression). The error term accounts for measurement errors and other unimportant, and thus negligible, factors considered to be noise. The correct form of the model should combine biological and epidemiological knowledge in the proper manner. Both identify the theoretical set of dependent and independent variables, link them within a biologically-based network of sub-processes properly accounting for interactions, non-linearities, feedbacks, and simultaneities. Specifically, in observational epidemiology, the relevant cancer risk factors (Table 2) must be accounted for (including the use of proxy variables). The model should not be limited to IR dose (*d),* Equation (1). If it is, the dose-response model is affected by the *specification error*: an issue regarding the incorrect model form and omitted risk factors (e.g., independent variables for other exposures and their mathematical form).

Using the LSS data, BEIR VII Phase 2 includes several models for solid tumors, where ERR depends on age at exposure, age attained, and time since exposure (Table 3). These models are prima facie underspecified: the estimates of the model's parameters will be biased and inconsistent. However, in animal bioassays *d* may be both necessary and sufficient – unlike epidemiology – because all other risk factors, e.g., from various exposures to behavioral, are controlled through the experimental protocol, animal husbandry, and so on.

The independent variables in Table 3 include city, *c*; sex, *s*; age, *a*; birth year, *b*; age at exposure, *e*; and *t* is time since exposure. For solid cancers, the (Poisson) model is: $ERR = \rho(d)_{\beta s}\exp(\gamma e)a^{n}$; ρ(d) is specified in different ways, including a threshold, but is generally taken to be either linear (and hence $\rho(d) = \beta_s d$), or linear-quadratic in dose (BEIR VII, p. 143).

The practical aspect for being concerned with the specification error is that, as [19] comment, '*epidemiological observations … have serious disadvantages … they can seldom be made according to the strict requirements of experimental science and therefore may be open to a variety of interpretation*' points to the need for careful causal analyses based on statistical methods. Regarding uncertainty stated as upper and lower confidence limits [20], discuss inference based on the linear RR model. They note '*that the distribution of the maximum likelihood estimate of β may be highly skewed, and that confidence intervals based on the estimates of the asymptotic standard error … can be seriously misleading*'. We concur with [21] statement that '*causal inference in epidemiology is better viewed as an exercise in measurement of an effect rather than as a criterion-guided process for deciding whether an effect is present or not*'. To advance the discussions, Table 4 includes steps towards formulating epidemiological causation, based on observational data, as statistical causal associations. Fig. 5 suggests the conceptual steps for developing a disease-specific model based on observational studies such as those discussed in this paper.

A well-specified epidemiological dose-response model also requires the following:

i) Development of the mathematical expressions that produce a mechanistic description of biological processes intervening between exposure and response, from different biological units and events, and causally relate dose (or dose rate) to specific cancers (e.g., lung cancer being different from leukemia).
ii) Accounting for directly contributing risk factors, ranging from environmental to behavioral (e.g., air and water pollution, smoking, alcohol consumption, poverty, etc.) affecting humans. For instance, the SES (socio-economic variables) of the correctly specified model would account for poverty. A simple dummy variable, could roughly account for poverty by stratifying above and below poverty level measured by income per capita. Therefore, by combining this type of information with experimental findings, such as non-linear biological processes from molecular events to whole organ response, results in a causal mathematical expression that *specifies* the form of the dose-response model, its variables, and coefficients.

2.2. Null hypotheses and statistical significance

When data are sampled from a population their analysis requires statistical methods. Two conditions are generally required: hypothesis testing and inference. For brevity, we focus on hypothesis testing. Under the null hypothesis, the coefficient (i.e., the statistical population parameter) of the linear model is zero; this hypothesis is tested (given the data) against either the one- or the two-sided alternatives: a positive or a negative estimate, given the data. The *p*-value (probability value) is the probability of obtaining either a positive or negative coefficient when the null hypothesis is true. Fisher stated that *the null hypothesis is never proved … but it is possibly disproved*. For example, Fisher's *significance* – i.e., that the results are not due to chance alone – varies from about *0.10* (*weak evidence* against the null) to *0.00010* or lower (which is increasingly *stronger evidence* against the null); e.g., a *p*-value *lower than 0.02 is strong evidence*. Neyman and Pearson developed type *1* and *2* error rates (for long-run frequencies). The *type 1* error (e.g., *false alarm*, e.g., set at *0.05* probability) occurs when the null hypothesis is incorrectly rejected; the *type 2* error (*failed alarm*, e.g., set at 0.20 probability) occurs when the *null* is incorrectly accepted. Specifically, if the confidence level adopted for the analysis is *0.95*, then there is a $\alpha = 0.05$ probability that the statistical result obtained is a false positive. In parallel, the probability of the *type 2* error is *β*; the *power* of a test is the probability of rejecting the null when it is false: *1 - β*. Interpreting *p*-values requires:

- A distribution function (which we take to be continuous and thus is a density function), whose area under its curve is exactly *1.00*.

Table 1
Modeling Issues (in bold) and assumptions from recent low dose IR epidemiological studies[a].

Study	Key Findings (emphasis added)	Key Assumptions	Modeling issues	Comments
[8]; IARC, occupational cohort with follow-up; mortality from leukemia and lymphomas; neutron doses	The estimated ERR is *evidence of a positive* ***association*** *between protracted low-dose radiation exposure and leukemia mortality*. The 6 positive slopes are ***highly imprecise***.	LNT as null hypothesis. Poisson regression with linear dose risk factors; LQ and Q; choice of model uses AIC. Cumulative exposure to IR regardless of dose rate; no other cancer risk factors accounted for. SES independent variables also modeled. Duration of employment. Lagged variables also used.	Inconsistent with phenomenological/mechanistic evidence for diseases considered. Choice of LQ contrary to AIC results, which points to Q. One-sided 90% confidence intervals. Confounders recognized but not accounted for. Heteroscedasticity affects 0–100 mGy response (RR < 1.00; lower CI bounds < 1.00). Some dose inappropriately thrown away (lagged)	INWORKS (France, US, UK) data-base. CML is statistically significant, other 6 cancer-specific models (e.g., AML and CML) are statistically insignificant. CLL has a significant negative slope. Pooling 3 leukemia yields significant results (individually AML and ALL are insignificant; CML significant). No scientific basis for throwing away radiation dose. LNT is inappropriate null hypothesis.
[11]; *BMJ*, IARC, mortality from occupational cohort with follow-up, solid cancers; external photon doses to colon.	Estimates *the* ***association*** *between protracted low dose exposure to ionizing radiation and solid cancer mortality*.	LNT as null hypothesis. Used ERR Poisson regression with country, age attained, sex, birth year SES, duration of employment, photon exposure. Some multiplicative variables, Q in dose also used.	Adjustments for smoking and asbestos. Some dose inappropriately thrown away (lagged). Their Fig. 1 suggests that heteroscedasticity affect the linear model. Cumulative exposure to IR regardless of dose rate.	INWORKS data-base. One-sided 90% CIs used. Cumulative colon dose appears be associated with statistically insignificant associations in 8 out of 10 cumulative doses. No scientific basis for throwing away radiation dose. LNT is inappropriate null hypothesis.
[12]; Ecological children study. Hazard rates from leukemias and CNS tumors, with follow-up.	The *study* ***suggests that background radiation may contribute*** *to the risk of cancer in children*	LNT as null hypothesis. Cox proportional hazards model (CPH) with background exposure to terrestrial and cosmic IR, air pollution (traffic) EMF, urbanization, SES, birth weight, birth order; trend analysis. The analyses may have statistical issues.	Inconsistent with phenomenological mechanistic evidence. 95% CIs show statistically insignificant results up to > 225 mSv (5 models) and 2 of these are significant above 225 mSv. One relationship has an average negative slope. Five of six HRs are insignificant, one has a p-value of 0.046, all have the lower 95% CL > 1.00.	Children < 16 years (Switzerland, 2 national census) exposed to background IR (gamma and cosmic rays, 0.9–1.8 mSv/year). It *should be interpreted with caution because of the uncertainties associated with using an ecological measure of dose.* [13], p. 77). LNT is inappropriate null hypothesis.
[14]; CT scans, retrospective cohort with follow-up; incidence and mortality from childhood leukemia and brain cancers	*Use of CT scans in children to deliver cumulative doses of about* 50 mGy *might almost triple the risk of leukemia and doses of about* 60 mGy *might triple the risk of brain cancer.*	LNT as null hypothesis, Poisson RR model. RR red bone marrow doses (mGy) from 0 to approximately 25 mGy are statistically insignificant. RR from brain dose approximately 20 mGy is also statistically insignificant.	No adjustments	UK, individuals < 22 years old. LNT is inappropriate null hypothesis.
[15]; record-based matched case-control, natural radiation and childhood cancers. Indoor exposure to gamma rays and radon.	… ***we cannot identify mechanisms by which confounding*** *might plausibly account for the magnitude and specificity of the results.* ***The association is therefore likely to be causal.*** Yet, it *is …* ***very difficult for an epidemiological study to detect the small relative increase in risk … produced by low level … radiation*** *against statistical fluctuations … and* ***confounding*** *factors*	OR via linear log-logistic model for dose. *12% excess relative risk … per millisievert of red-bone-marrow dose from gamma radiation; … association with radon was not significant … other childhood cancers … not significant for any radiation type. Excess risk … insensitive … adjustments for socio-economic status.* Also, used logistic model with dose and SES variables.	Two-sided CIs at 95%. Power is approximately 50%. Father social class at child birth implied from occupation. SES (by census ward): male unemployment, social class and proportion of household, non-car ownership. Radon and gamma rays do not act independently. Thus, invalid assumption used in data analysis.	Great Britain, match on national tumor registry. Incomplete SES variables measurements and changes over time (p. 22, 23). RR from time-integrated gamma rays and all cancers) = 1.03, (CI 95%): 1.00–1.07, p = 0.04, with leukemia RR = 1.09, CI 95% = 1.02–1.17, p = 0.01.
[16]; cohort, children CT scans	… ***confounding*** *… or* ***reverse causation*** *cannot be ruled out ….*	Possible misestimated exposures.	*SIR (SIR = O/E) was 1.72 (95% CI 0.89–3.01, O = 12), and for CNS tumors, the SIR was 1.35 (95% CI 0.54–2.78, O = 7).* O is observed, E is expected numbers.	Germany; cases stochastically linked to German Childhood Cancer Registry for Leukemia.
[17]; cohort, children, CT scans, follow-up; CNS cancers, leukemia, lymphomas.	***No significant excess risk was observed in relation to CT exposures.***	ERR estimation via Poisson regression. Short follow-up (~4 years). Accounted for 12 possible genetic factors and immune deficiencies (e.g., HIV). Additional variables include: sex, period of birth, attained age, time to entry in cohort. Doses were lagged.	Predisposing disease were identified and included in the analysis.	France; other cancers also included (e.g., retinocytoma). Dose ranged from < 5 mGy to > 100 mGy. These *are preliminary estimates of risk*.
[18]; all cancers, CT scan, children and teens, cohort with follow-up.	*The* ***increased incidence of cancer*** *after CT scan exposure in this cohort was* ***mostly due to irradiation.***	RR increases as a function of the number of scans. *The RR increased significantly for many types of solid cancer (digestive organs, melanoma, soft tissue, female genital, urinary tract, brain, and thyroid); leukaemia, myelodysplasia, and some other lymphoid cancers*	32/64 statistically insignificant results (95% confidence interval); cancers associated with CT scans are statistically significant. Sample sizes from 3 (brain, thyroid) to 1038 (other solid cancers). IRR is stratified by cancer site, one-year lag, age, sex, birth year.	Australia; individuals < 20 years old. Younger ages more susceptible.

[a] OR is odds ratio, RR is relative risk, SIR is standardized incidence ratio, CT is computed tomography, SES is socio-economic, HR is hazard rate.

Table 2
Known risk factors for solid cancers associated with IR exposure.

Organ-Specific Cancer	High risk factors	Environmental risk factors	Other risk factors
Stomach	Diet (salty, smoked, fatty); low fruit diet, aflatoxin, familial history, smoking, stomach infections, pernicious anemia, polyps, blood type, EBV, obesity.	Occupational exposure to chromium, asbestos, solvents, lead.	Genetics, A blood group.
Colon	Diet (red meats), familial history, genetics, inflammatory bowel disease, polyps, ethnicity.	PAHs.	Smoking, alcohol consumption.
Lung	Smoking, diet (red meat), familial history, previous lung diseases.	Naturally occurring radon, exposure to As, asbestos, diesel fumes, silica, paints solvents, chromium, nickel; general air pollution, beryllium, TCDD, $PM_{2.5}$, PM_{10}, As.	Mineral oils.
Liver	Diet (alcohol consumption), lupus, smoking, chronic infections (e.g., HVC, HBV), cirrhosis, inherited diseases, aflatoxin, ethnicity.	As, VC, PCBs, parasites.	None significant, (see VC and PCBs).
Breast (female)	Familial history, genetic predisposition, ethnicity, race, breast density.	Occupational exposures.	Alcohol use, hormones, obesity, null-parity.

Table 3
Specification of BEIR VII Phase 2 dose-response models (LSS Data).

Cancer D-R Model	Model Form	Incidence	Death (counts)	Competing Causes	Comment
Solid Cancers	$ERR = \beta_s dExp(\gamma e)$	Y	Y	Omitted	LSS study; β_s = risk of exposure at age 30. Dose in Sieverts.
Solid Cancers	$ERR = \beta_s dExp(\gamma e^*)a^{\eta}$	Y	Y	Omitted	LSS Study; dose in Sieverts
Solid Cancers	$ERR = \beta_s dExp(\gamma e^* + \eta \log(a/60))^a$	Y	Y	Omitted	LSS Study; Site-specific cancers (e.g., stomach, breast, liver, etc.) See Tables 12B–5A BEIR VII p. 303).
Leukemia	$ERR = \beta_s d(1+\theta d)Exp[(\gamma f(e) + \delta g(t) + \phi f(e)g(t)]$	Y	N	Omitted	CLL not included

The specification of the functions in these models include g(t) = log(t) and g(t) = t, f(e) = (e − 30)/10 for e < 30 and 0, for e = 40; *d* is dose.

Table 4
Key steps towards developing causal associations from observational data in epidemiological studies.

- *Identify and formalize causal mechanism(s) and paths.* Show how changes propagate via one or more causal paths to produce adverse, protective, or other effects. A causal path is a sequence of steps in which completion of the earlier steps creates conditions that trigger or increase occurrence rates of subsequent steps. Such steps may be identified from experimental data, applying generally accepted physiological and other scientific laws or known relationships. A priori, correlations are not causal; see point i) below.
- *Identify statistically significant exposure-response causal association.* Demonstrate that there is a non-random positive statistical association between exposure histories or events and adverse human health consequences, see point ii) below. Associations between time series (such as cross-correlations) may not be causal associations.
- *Eliminate confounding as a possible cause of the association.* Show that cause is not due to or explained by other factors, such as differences in lifestyle, age, or confounders.
- *Eliminate biases from sampling, information collection, and modeling choices.* Show that the association is not explained by selection bias (study subjects or controls) or how information about them was collected and analyzed.
- *Test and confirm hypothesized causal ordering and conditional independence relations between variables.* Show that response is not conditionally independent of its direct causal predecessors (e.g., exposures and specific risk factors), but that it is conditionally independent of more remote causal risk factors or effect modifiers.
- *Model predictions.* Confirm that changes in the levels of the causal variables (e.g., exposures) are followed by the predicted changes in the levels of the effects or outcomes.

- The choice of one-sided or two-sided test itself (the two-sided test rules in two alternatives; the one-sided test rules in only one alternative).
- A test statistic (e.g. *z*-score), to determine the *p*-value calculated from the data.
- A theoretical (or decisional) *p*-value (the *choice* of the probability number that rules out the null as being due to chance).

As Sterne and Davey Smith [22] state, the use of secondary data can create biases:

> If only positive findings are published, then they may be mistakenly considered to be of importance … The high volume and often contradictory nature of medical research findings, however, is not only because of publication bias. A more fundamental problem is the widespread misunderstanding of the nature of statistical significance.

Balancing false positive and false negative probabilities must be explicit in any analysis of the statistical significance of estimated results because changing the model's risk factors (e.g., age intervals, exposure variables, for a constant sample size) affects those probabilities.[4] The tests concern: i) single hypothesis under the null (the probability of rejecting the j-th null hypothesis, $h_{j0}|true \leq \alpha$); ii) multiple null hypotheses; and iii) combinations of multiple null hypotheses, some of which are false and some of which are true. A method for assessing multiple sampling evidence is Bonferroni's test.[5] Its results are conservative: they understate statistical significance.[6] Remedies for the issues that arise from multiple comparisons are listed in Table 5.

[23] discusses the bias that occurs when statistically significant hypotheses (forming the global null) are reported, while the individual, insignificant results are not (Table 6). He corrects multiple testing bias

[4] Assuming *m* probabilistically independent comparisons, the actual type *1* error rate is $\alpha^* = 1 - (1 - \alpha)^m$, with α being the nominal error rate under m = 1 comparisons (the theoretical model). If the comparisons are correlated, then $\alpha^* \leq 1 - (1 - \alpha)^m$. Thus, if m = 4, the actual error rate is 0.226, rather than 0.05. A simple way to deal with this issue would be to use the (conservative) Bonferroni inequality: $\alpha^* = 1 - (1 - \alpha)^{1/m}$, which can be reduced to $\alpha^* \leq 1 - (1 - \alpha)$ with $\alpha^* = \alpha/m$, and solving for α. This presumes that the number of comparisons is set in the protocol before conducting the study.

[5] Tukey [58] discusses the effect of heterogeneity on the F-test and Hayter and Hsu [59] the confidence intervals.

[6] Bonferroni's α is calculated as the α_{FEW}/I, $\alpha_{FEW} \leq 1 - (1 - \alpha)^i$. It has low power because it assumes that h_i is true but does not account for possible correlations and hence it overcorrects for the type 1 error. That is, the number of false positives increases depending on the correlations between the hypotheses tested. Bonferroni's inequality is defined as $\left[pr\left[\bigcup_{i=1}^{n} Uh_i \leq \sum_{i=1}^{n} p_{\eta}(h)\right]\right]$.

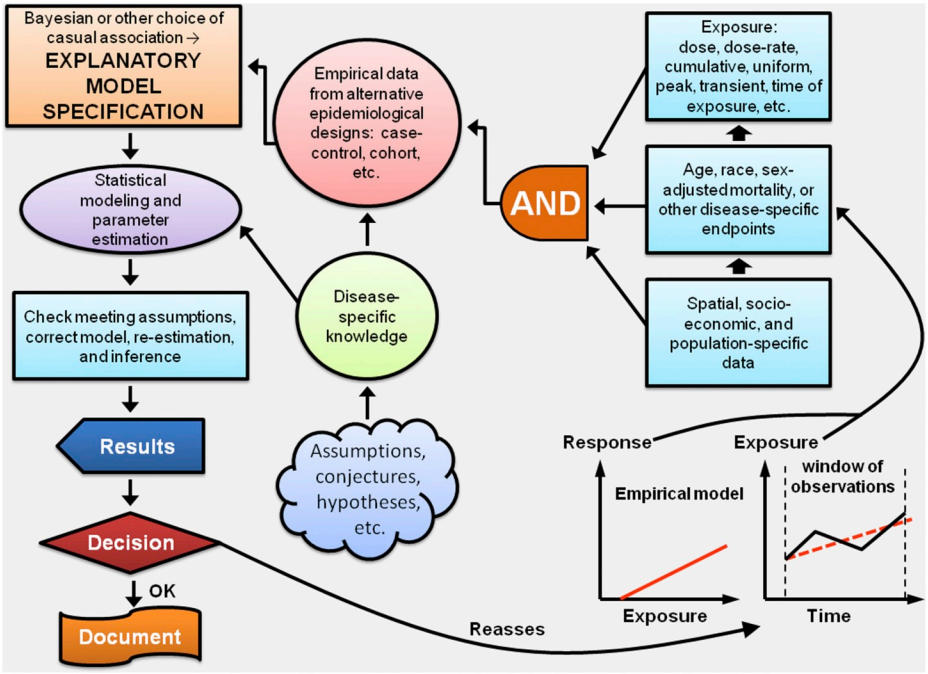

Fig. 5. Conceptual exposure-response model building (sub-processes, e.g., pharmaco-kinetic and pharmaco–dynamic, omitted for brevity). The stopping rule for the loop is unspecified because it depends on the disease-specific endpoint (e.g., mortality), theoretical, and empirical knowledge.

Table 5
Multiple comparisons issues and remedies.

Issue	Characteristics	Remedy
Stratification	Multiple comparison problem: inflates the false positive probability.	*Analytical*: Specify the strata, sub-groupings, or hypotheses before the statistical analysis.
Repeated analyses	Multiple pair-wise comparisons.	*Analytical*: Specify a single, theoretical model for estimation and use *F*-test or other global test of significance.
Interpretation of significance	*Statistical* significance ≠ *theoretical* significance.	*Semantic*: Use the correct and appropriate phrase by discriminating between these uses.
Multivariate modeling	*p*-values are inflated by multiple testing.	*Analytical*: Given a class of models, use Bayesian modeling to select the model via the maximum of the posterior distribution.
Several simultaneous statistical tests	In hypothesis testing, inflated *p*-value.	*Analytical*: Nominal probability value (e.g., $\alpha = 0.05$) is increased as the power of the number of tests performed.

Table 6
Inference related issues from sampled data affecting the *LNT* epidemiological literature (adapted from Ref. [23]).

Issue	Comment
The smaller the studies conducted in a scientific field, the less likely the research findings are to be true.	Small samples are less statistically powerful than large samples.
The smaller the effect sizes in a scientific field, the less likely the research findings are to be true.	Small sample are less likely to detect a true effect of relatively small size.
The greater the number and the lesser the selection of tested relationships in a scientific field, the less likely the research findings are to be true.	A hypothesis generating modeling is unlikely to yield causal results.
The greater the flexibility in designs, definitions, outcomes, and analytical models …, the less likely the research findings are to be true.	Model flexibility can result in statistical biases; bias-variance trade-offs become relevant.

by assigning low probabilities to each independent variable [24]. state:

> Consider an unlikely hypothesis, with a prior probability of only 1%. Under the listed assumptions, obtaining a p-value of exactly 0.05 would move this 1% prior probability up to only a 2.6% posterior probability, P ≤ 0.05 would raise it to 14%, P = 0.001 would raise it to 50%, and "P < 0.001" would raise it to 73% - a quite respectable level. Even given that these posterior probabilities represent maximum values without consideration of weaknesses in study design, this does show that an implausible alternative hypothesis can be made plausible if the evidence is strong enough. But that cannot happen if all p-values below a conventional threshold like 0.05 are treated as evidentially equal.

Ioannides' comment that increasing the number of studies increases the number of false positive and false negatives should be kept in mind. However [24], find that '*this is not true as a proportion of the total, which is the probability that a given finding is false, i.e. the predictive value. If the number of positive studies is held constant while the total increases, the predictive value of all studies combined decreases, albeit not because the positive predictive value of any positive study decreases, but because the negative predictive value of all the non-significant studies outweighs the positive predictive value of the significant ones*'. Table 7 includes other biases affecting the determination of epidemiological causal associations.

Table 7
Selected biases affecting the determination of epidemiological causal associations [25]; references therein.

Types of Biases	Account for These Types of Bias
1) Modeling Biases	**Discussions**
Variable selection bias (including selection of covariates included in the model)	Bootstrap variable selection, Bayesian model averaging (BMA), model cross-validation for variable selection.
Omitted explanatory variables (including omitted confounders and risk factors)	Include potential confounders in an explicit causal graph model; test for unobserved latent variables,
Variable coding bias	Use automated variable-coding methods (e.g., classification trees). Don't discretize continuous variables.
Aggregation bias (e.g., Simpson's paradox)	Test hypothesized relations at multiple levels of aggregation. Include potential confounders in an explicit causal graph model.
Multiple testing or comparisons bias	Use step-down methods to adjust *p*-values without sacrificing power.
Choice of exposure, dose, and response metrics	Use multiple exposure indicators (e.g., concentration and time). (Don't combine.) Define responses as survival functions and/or transition rates among observed health states.
Model form selection bias and uncertainty about the correct model for exposure-response.	Use flexible non-parametric models (e.g., smoothers, wavelets) and Bayesian Model-Averaging. Report model diagnostics and sensitivity analyses of results.
Missing data adjustments	Use data augmentation, EM algorithms, MCMC algorithms.
Measurement and misclassification errors for the explanatory variables	Use Bayesian measurement error models, data augmentation, EM algorithm, and other missing-data techniques
Omitted heterogeneity in individual response probabilities and covariates	Use latent variable and mixture distribution models, frailty models of inter-individual variability
Interpretation and reporting of results	Report results (e.g., full posterior PDFs) *conditioned* on choices of data, models, assumptions, and statistical methods.
2) Selection Biases	**Discussions**
Sample does not represent population for which inferences are drawn.	Randomly sample *all* cohort members if possible
Data set (i.e., selection of a subset of available studies may affect results)	Use meta-analysis to show sensitivity of conclusions to studies included/excluded. Use causal graph models to combine diverse data sets
Health status confounding or hospital admission bias (as well as referral and exclusion)	• Use prospective cohort design • Use population cases and control
Selective attrition/survival (e.g., exposure affects attrition rates). Differential losses to follow-up.	May use reconstruction of events through indirect interviews Develop counter-factual survival curves
Detection and surveillance	Match cases to controls (or exposed to unexposed) subjects based on cause of admission.
Membership (e.g., lifestyle, socioeconomic)	Use multiple comparison cohorts. Hard to control in observational studies.
Self-selection, volunteer bias	Achieve high response rate. Compare respondents with sample of non-respondents

3. Biological events likely to affect the form of the LNT

Molecular epidemiology combines quantitative methods with biochemical, molecular and other data. These can provide the: i) precise biological unit at risk (from proteins to cells); ii) measurement of the magnitude of an effect; iii) description of biochemical processes leading to a pre-disease stage; and iv) data to be used in stochastic and non-stochastic dose-response models. Markers, such as DNA-adducts, can describe how a carcinogen acts with the DNA or a protein, switches on a gene, and so on. The NCRP (2015, p. 2) states that '*an essential problem is the lack of bioindicators … specific to radiogenic disease* and whether *genetic instability transfers from normal to cancerous cells or from pre-neoplastic to cancerous'* and that it is difficult to identify human tumors and radiation-related tumors because tumor pathways may not conserve across species. In part, this is a result of the '*lack of known tumor biological indicators that are specific to radiation exposure'* (NCRP, p. 38). Indicators of DNA damage and repair, mutations, genomic instability, adaptive response, bystander effects and other, inform the specification of the dose-response (Table 8).

For NCRP (2015, p. 10), biologically-based dose response (BBDR) models (such as [26,27]; and others) *may be of limited value for reliably predicting cancer risk. The reason is that they do not incorporate biological data other than generalized mutagenic evidence supporting the MS* (multi-stage) *model.* [28] exemplifies the current view on mechanistic reasoning and the use of epidemiological results at high doses to extrapolate to low doses. He states that the *LNT* (p.276; citations omitted) '*is now made unlikely by the observation of low-dose specific biological responses, which unambiguously demonstrate the failure of extrapolation of radiation effects per unit dose from high to low doses'*. He uses 18 studies (yielding 54 data points) published in peer-reviewed journals from 1986 to 2012, concerning molecular and cellular effect of acute and low-dose exposure to x or γ-rays. Adaptive protection at the cellular, cancer, immune response, bystander damage, and enzyme inactivity occurs at doses between 0 and $>$ 600 mGy. In transgenic mice, damage occurs at two dose levels: 0.01 mGy and 100 mGy; some damage was apparent in non-transgenic mice at 0.01 mGy, although these animals exhibited protective response or low damage. Thus, while the initial damage at the molecular level is proportional to dose, the affected biological unit at risk of propagation of that initial damage exhibits a non-linear dose-response. This is due to DNA repair, bystander effect, genomic instability, and adaptive protection mechanisms. Specifically, bystander effect and genomic instability contribute to damage but adaptive protection reduces it; the time-windows are measured in hours or days, extending to approximately a year, depending on the specific mode of action [29]. Sanders (2010; p. 279) had earlier commented that '*the measured degree of protection is related to the enormous efficiency with which the body protects itself against the development of malignancy'* [30]. argues that the *LNT* is incorrect because of adaptive protection, enhanced immune response, protective bystander effects, and aspects of genomic instability [31]. further notes that targeted versus non-targeted events should be accounted for, as should cascading events (e.g., genomic instability → gene mutations).

We summarize [32,33] discussions in Table 9, within the context of chemical carcinogenesis, rather than IR. Bogen and Crump are concerned with the *additive to background*, AB, theory. For example [32], discusses cellular experiments resulting in apoptosis and other, often non-linear processes leading to cellular cytotoxicity. Those are modeled by cumulative distribution functions (e.g., using log-normal distributions which assume multiplicative events). We concur with [32] (citations omitted) conclusion that '*importantly, LNT-like dose-response relationships based on epidemiology studies may be an artifact due to systematic confounding effects associated with exposure measurement errors that obscure underlying nonlinear relationships for population dose response'*.

Table 8
Events on selected biological units at risk from IR (A) or chemical (B) exposures: relevance to developing biologically motivated low dose-response cancer models[a].

A

Events	Damage or Outcome	Example of Repair or Other
DNA Damage	SSB, DSB, and so on.	Base excisions, mismatch, recombination.
Signaling from normal cells	DNA damage.	Check-point pathways, signal transduction, cell arrest, cell death.
Chromosomal Damage	Aberrations, Rearrangements.	Homologous recombination; direct repair.
Mutations	Genetic point or other, base-pair substitutions, insertions and deletions.	Reductions in frequency.
Genomic Instability	Increased gene copies, altered chromosomal arrangements, cell killing.	Adaptive response.
Bystander effects in non-exposed cells	DNA damage, chromosomal instability, transformations.	Apoptosis, delayed tumor cell growth.
Genetic susceptibility of exposed cells	Excess spontaneous cancers and increased probability of disease.	Low probabilities in heterogeneous populations.
Cellular adaptive response; Overcompensation	In vitro, *in vivo* cellular responses at low and high (subsequent) dose.	Reduced frequencies of phenotypic alterations in high background cells, apoptotic selective cell removal. Enhanced state rather than damage.

B

Class of Events Leading to D-R Models	Mechanism: Exposure (- negative effect; + positive effect)	Example of Outcome or Process	Main References
Genotoxic mutations	Mutations leads to (−) malignant phenotype.	Formation of DNA adducts.	[50,51]
Genomic instability	Recessive (2 (−) mutations); dominant gene (1 (−) mutation).	Immuno-surveillance; Microsatellite instability, mismatch repair.	[52,53]
Non-genotoxic clonal expansion	Epigenetic (−) functional changes.	Methylation of cytosine, histone acetylation.	[54]
Darwinian cell selection	Selective advantage, fitness (+) of mutated cells.	Mutation 1→ clonal expansion → mutation 2 → phenotype.	[55,56]
Tissue-specific structural, biochemical changes	Vascularization (−) of different types.	Micro-environmental morphogenesis.	[57]

[a][6]; [11,13,40–45].

Table 9
Discussions of [32] and Crump [33] regarding three alternative dose-response models [34].

Alternative	Description	Findings by Ref. [32] nd Crump [33]
Model 1	Threshold dose response at the individual level, but linear at the population level due to *substantial* human *individual heterogeneity of susceptibility* (SIH) such that their aggregate is *LNT*.	[33] proves that the SIH implications for the *LNT* are *generally false*, as earlier put forth by Ref. [32].
Model 2	Threshold response at individual level, non-linear at population level, not yielding an *LNT*.	None remarkable.
Model 3	Linear response at the individual level, linear at the population level; characterized by linear MOA, and for non-linear MOA when response is additive to background, yielding a *LNT*.	[32] finds that the AB argument *is either false or meaningless*, but Crump [33] limits the extent of this criticism to it not being correct when *background response occurs by a mechanism that is completely independent of that of the substance of interest* [32]. criticism of the AB is appropriate because, as Crump [33] states: *additivity to background, without additional conditions, does not assure that the low dose guaranteed by the AB can be well-predicted by data at high doses* [32]. issue *that the slope of the dose-response at d = 0 may not be well-predicted by the trend of the dose-response at higher doses* [33], is based on quasi-thresholds, which [33] finds to be an artifact of scaling the plots obtained by Ref. [32].

Statistical parameter estimation uses sample data for the dependent and independent variables to obtain numerical values for the parameters (the mathematical coefficients) of the model. It must account for *confounding variables*: independent variables not on the path between a risk factor and the health outcome, but that influence both. In estimation, the error term is associated with the dependent variable. However, errors also affect the independent variables in the model. Not accounting for the errors in the independent variables produces inconsistent parameter estimates [35]. The *error-in-variables* model [36,37] could be used to investigate Bogen's observations. However, the estimation methods are more complicated [38] than those used to estimate the parameters (e.g., the maximum likelihood) of a more fully specified exposure-response model.

4. Towards resolving the LNT controversy: contrary views point to a solution

There are two prominent science-policy views about the *LNT*. One is BEIR VII Phase 2 (US NAS) finding that observational epidemiological studies are inherently limited, as are *in vivo* studies (p. 245, emphasis added by underscoring):

> … It is abundantly clear that direct epidemiologic and animal approaches to low-dose cancer risk are intrinsically limited in their capacity to define possible curvilinearity or dose thresholds for risk in the range 0–100 mSv.

The other is the French Academies' view (2005):

> For doses above approximately 200 mSv, epidemiological data permit to establish with fair accuracy the relationship between dose and carcinogenic effect. However, for low doses (below 200 mSv) and *a fortiori* below 20 mSv generally encountered within the context of radioprotection, epidemiology can neither confirm nor refute the existence of an increased incidence of cancer.

Yet, important differences between these two institutions become apparent. Here, the overlap between the literature in both studies is only 68 papers; the French Academies used 306 papers and BEIR VII Phase 2 used 1386, for a total of 1760 papers; the French Academies focusing on biological mechanisms, in particular adaptive protective response.[7] In the second, the French Academies state that, although their report is about IR:

> … it is apparent that most of its conclusions can also be applied to other physical (U.V. radiation) and chemical (genotoxic) carcinogenic agents, for which often, for administrative reasons there is also a tendency, to apply a linear no-threshold relationship …. For each toxic effect, there are specific defense mechanisms …. The outcome depends on the balance between these two types of reaction. If the dose is low and defenses are sufficient, there will be no toxic effect. If the dose is high, and defense reactions are overwhelmed … a toxic effect emerges and becomes proportional to the dose.

Specifically, regarding the effects of low versus high exposures to IR, this institution states that:

> At low doses and low dose rates of ionizing radiation, the pro-apoptotic effect dominates and the damaged cells, of which there are only a few, can be eliminated or controlled. But at doses in excess of 0.5 Gy with a high dose rate, the greater number of mutant cells and the accumulation of mutations, the tissue disruption and above all the proliferation of the surviving cells to compensate for the death of a high proportion of the cells allow some cells to escape from these controls, which are intended to maintain tissue integrity and to regulate proliferation.

Third, the French Academies (citations omitted) supports our concern with model specification:

> … the absence of any correlation between the dose received and all the other potential risk factors (such as tobacco) should be established. If such factors are present, they must be taken into account by appropriate statistical methods. This point is particularly important with regard to the study of low doses, because the specific effect of the confounding factors can be much greater than the effect of radiation …. For example, in a study investigating the risk of lung cancer due to radon in homes, not taking smoking into account would make the results impossible to interpret … accurate information must be available about all exposures to ionizing radiation, including those unrelated to the source of irradiation being investigated. This is difficult, given the frequent and possibly repeated exposures to small doses of radiation: natural irradiation (differences of natural irradiation can reach 20 mSv/year), X-ray examinations, air travel …. They may introduce biases even when they are smaller than the irradiation investigated.

To the contrary, BEIR VII Phase 2 (2006, p. 263) finds that:

> Biologically based models have not been employed as the primary method of analysis in this report for several reasons. The mechanisms of radiation carcinogenesis are not fully understood, which makes the development of a fully biologically based model difficult. The data required for a biologically based model, such as rates of cell proliferation and mutation, are also generally not available. The availability of empirical risk models that provide a good description of the available data on radiation and cancer permits the preparation of useful risk projection.

Those *empirical risk models* are mostly observational epidemiological

[7] Personal communication, Nov. 14, 2017, by Dr. Tony Brooks.

studies. A suitable test of the usefulness of the *LNT* for science policy should be accurate causal predictions; yet, how can this test be conducted if the *mechanisms of radiation carcinogenesis are not fully understood*? Incorrectly specified *empirical risk models* should not be used to forecast outcomes.[8] Model prediction, rather than description, correctly informs policy science. BEIR VII Phase 2 arguments seem internally contradictory (p. 263, titles in bold added):

i) **Description Is Insufficient for Policy Science Causation** – A BEIR VII Phase 2 argument is that *'whereas empirical approaches to risk modeling rely on statistical models to describe data, biologically based models depend on fundamental assumptions regarding the mechanisms of radiation carcinogenesis. The parameters created by modern biologically based risk models have a direct biological interpretation, provide insight into cancer mechanisms, and generate substantive questions about the pathways by which exposure to ionizing radiation can increase cancer risk.'*

ii) **Prediction Is Necessary for Policy Science Causation** – BEIR VII Phase 2 argument is that *'biologically based models have not been employed as the primary method of analysis in this report for several reasons. The mechanisms of radiation carcinogenesis are not fully understood, which makes the development of a fully biologically based model difficult. The data required for a biologically based model, such as rates of cell proliferation and mutation, are also generally not available. The availability of empirical risk models that provide a good description of the available data on radiation and cancer permits the preparation of useful risk projection.* We disagree with the last statement because it is inconsistent with sound modeling by conflating description with prediction. For example, the statement ignores the bias-variance effect whereby a polynomial (or a Fourier series) can perfectly fit a scatter of data, such as those shown in Figs. 1–4. However, the predictions from those polynomials are fundamentally different from what the trends in the data allows to predict with some reasonable confidence.

BEIR VII Phase 2 (p. 141) states that the evidence from the LSS for effects at low dose is that:

> Estimates of linear risk coefficients tend to be driven by doses that exceed 0.5 Gy; although estimates based only on survivors (of the LSS) with lower doses can be made, their statistical uncertainty is considerably greater than those that include survivors with higher doses. Even at higher doses, data are often inadequate for evaluating risks of cancers at specific sites, especially those that are not common …

Hence, the *LNT* epidemiological model *is* a conjecture. Paradoxically, we find support for this conclusion in BEIR VII Phase 2 itself (p. 245):

> … human data well illustrate the problems of limited statistical power that surround epidemiologically based conclusions on the shape of the low dose-response for radiation cancer risk and how it might vary between tumor types. … It is abundantly clear that direct epidemiologic and animal approaches to low-dose cancer risk are intrinsically limited in their capacity to define possible curvilinearity or dose thresholds for risk in the range 0 – 100 mSv.

BEIR VII Phase 2 basic mechanistic explanation for the *LNT* seems simplistic (p. 245):

> Whatever molecular mechanism is envisaged for radiation, at very low doses (e.g., 0–5 mGy low LET), increases in dose simply increase the probability that a given single cell in the tissue will be intersected by an electron track which will have a nonzero probability of inducing a biological effect. Therefore, at these very low doses, a linearity of response is almost certain.

We suggest five qualitative criteria to deal with the problem that [39] describes as *'no evidence for or against a substantive claim (theory) can be secured based on a statistically misspecified model* of dose-response for cancer at low doses'. These are:

I. Independence and unbiasedness of those replicating the results – given the same research protocol.
II. Rejection of *dogmatism.*
III. As the number of independent replications increases, their result should tend towards the true result.
IV. The scientific information and knowledge base used in developing the dose-response model and its analyses should be publicly available so that any replication can be – at least in principle – performed and assessed by interested stakeholder.
V. Any disease-specific finding of application of an *LNT* must be proven to be consistent with both explanation and prediction through validated theoretical and empirical mechanistic reasoning.

5. Conclusions

The IR-related LNT is a conjecture for humans, a testable hypothesis for lower species and other biological systems, and a science-policy tool for regulating exposure. Our analyses suggest that the model at low doses is no model at all: aggregating epidemiological studies yields results that are statistically insignificant. When non-linearity occurs, it is statistically significant at high doses beyond the concern of the *LNT*. The key question we address is whether the choice of the *LNT* at low doses, given IR epidemiological information, is correct. The answer is: *no,* the *LNT* remains a conjecture. To justify this answer, we discussed critical causally associative – *causal* in short – modeling issues and biological and epidemiological evidence, focusing on the latter. Specifically, we conclude that:

1) The biological-mechanistic evidence that should be reflected in the *LNT* is extensive; limiting its inclusion in formalizing the relationships between IR and ERR contributes to the specification error. The mathematically causal expression should account for fundamental biological mechanisms such as: adaptive protection, enhanced immune response, protective bystander effects, and aspects of genomic instability. The set of independent variables for the cancer of concern should be extended to include: i) other exposures; ii) behavioral, socio-economic, and other risk factors.
2) Aggregate epidemiological results indicate low-dose thresholds – as statistically insignificant responses – rather than *LNT* behaviors roughly between 0 and 100 mSv. Given the empirical epidemiological evidence, causally associative epidemiological models should include a threshold by constraining estimation according to the empirical results shown in this paper.
3) It is incorrect to assume that biological mechanisms at high doses can be extended through an interpolation (to 0, background risk) since molecular and cellular responses to high and low doses are different with the high doses responding to damage and the low doses initiating many repair processes. In addition most epidemiological studies at low doses are statistically insignificant while being significant at high IR doses.
4) The assertion of linearity at low doses conflates evidence at high doses with policy ease.

[8] Forecasts are response values obtained, given a model, outside the dose sample and include the uncertainty about those numbers. For any best-fitted line to the sample data, the uncertainty about the fitted line consists of non-linear curves (at selected level of confidence) about that fitted curve. Although the LNT is a linear function at low doses, these symmetric non-linear bounds increase and diverge at increasing rates from the central estimate values of the model's parameter representing its slope [60].

These conclusions result from assessing such statistical issues as: i) model specification, ii) confounding factors, and iii) multiple testing bias. Modeling, inclusive of theoretically relevant sets of dependent and independent variables, links them within a biologically plausible network, accounting for non-linearities, feedbacks, temporal and other delays, and possible simultaneities. The statistical significance of epidemiological results, an important aspect of assessing thresholds at low doses, can be associated with an often-unwarranted emphasis on *p*-values, as discussed. Empirically, the upper and lower confidence limits must also be included. These identify lack of significance and thus no effect under the null hypothesis, even though the magnitude of the increased risk is numerically positive (e.g. ERR > 0).

Regarding the science-policy aspect of the *LNT*, BEIR VII Phase 2 has supported the *LNT* through conjectural reasoning and conflates science-policy with causation by appealing to vague statements that cannot be formally reconciled with scientific evidence. The statistical analyses conducted by BEIR VII Phase 2 use misspecified models that do not include, but rather seem to force, linearity either via the linear or the linear-quadratic specification. We find that the entirely different conclusions reached by the US National Academies of Science (BEIR VII Phase 2) and the French Academies of Science and Medicine regarding the epidemiological *LNT* can be resolved through correctly specifying a dose-response model that integrate both mechanistic and epidemiological knowledge. Formal aggregation of subjective expert judgments may bridge those differences and better inform science-policy about the risks associated with low levels of IR exposure.

Declarations of interest

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Appendix A. Supplementary data

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References

[1] NAS/NRC, Health Risks from Exposure to Low Levels of Ionizing Radiation, BEIR VII, Phase 2, National Academy Press, Washington DC, 2006.
[2] A. Aurengo, D. Averbeck, A. Bonnin, B. Le Guen, R. Masse, R. Monier, M. Tubiana, A. Valleron, F. de Vathaire, Dose-Effect Relationships and Estimation of the Carcinogenic Effects of Low Doses of Ionizing Radiation. Executive Summary, French Academy of Sciences, French National Academcy of Medicine, 2005.
[3] B.L. Cohen, Test of the linear-no threshold theory of radiation carcinogenesis for inhaled radon decay products, Health Phys. 68 (2) (1995) 157–174.
[4] A.K. Thompson, M.A. Coplan, J.W. Cooper, P.P. Hughes, R.E. Vest, C. Clark, Observation of the (3)He(n, tp) reaction by detection of far-ultraviolet radiation, Journal of research of the National Institute of Standards and Technology 113 (2) (2008) 69–78.
[5] J.S. Thompson, R. Weseman, F. Rochling, W. Grant, J. Botha, A. Langnas, D. Mercer, Radiation Therapy Increases the Risk of Hepatobiliary Complications in Short Bowel Syndrome, Nutrition in Clinical Practice : Official Publication of the American Society for Parenteral and Enteral Nutrition 26 (4) (2011) 474–478.
[6] D.J. Brenner, R. Doll, D.T. Goodhead, E.J. Hall, C.E. Land, J.B. Little, J.H. Lubin, D.L. Preston, R.J. Preston, J.S. Puskin, et al., Cancer risks attributable to low doses of ionizing radiation: assessing what we really know, Proc. Natl. Acad. Sci. U. S. A 100 (24) (2003) 13761–13766.
[7] O. Gregoire, M.R. Cleland, Novel approach to analyzing the carcinogenic effect of ionizing radiations, Int. J. Radiat. Biol. 82 (1) (2006) 13–19.
[8] K. Leuraud, D.B. Richardson, E. Cardis, R.D. Daniels, M. Gillies, J.A. O'Hagan, G.B. Hamra, R. Haylock, D. Laurier, M. Moissonnier, et al., Ionising radiation and risk of death from leukaemia and lymphoma in radiation-monitored workers (INWORKS): an international cohort study, The Lancet Haematology 2 (7) (2015) e276–281.
[9] E.J. Grant, A. Brenner, H. Sugiyama, R. Sakata, A. Sadakane, M. Utada, E.K. Cahoon, C.M. Milder, M. Soda, H.M. Cullings, et al., Solid cancer incidence among the life span study of atomic bomb survivors: 1958-2009, Radiat. Res. 187 (5) (2017) 513–537.
[10] A.R. McLean, E.K. Adlen, E. Cardis, A. Elliott, D.T. Goodhead, M. Harms-Ringdahl, J.H. Hendry, P. Hoskin, P.A. Jeggo, D.J.C. Mackay, et al., A restatement of the natural science evidence base concerning the health effects of low-level ionizing radiation, Proceedings Biological sciences 284 (1862) (2017).
[11] D.B. Richardson, E. Cardis, R.D. Daniels, M. Gillies, J.A. O'Hagan, G.B. Hamra, R. Haylock, D. Laurier, K. Leuraud, M. Moissonnier, et al., Risk of cancer from occupational exposure to ionising radiation: retrospective cohort study of workers in France, the United Kingdom, and the United States (INWORKS), Br. Med. J. 351 (2015) h5359.
[12] B.D. Spycher, J.E. Lupatsch, M. Zwahlen, M. Roosli, F. Niggli, M.A. Grotzer, J. Rischewski, M. Egger, C.E. Kuehni, G. Swiss Pediatric Oncology, et al., Background ionizing radiation and the risk of childhood cancer: a census-based nationwide cohort study, Environ. Health Perspect. 123 (6) (2015) 622–628.
[13] UNSCEAR, Sources, Effects and Risks of Ionizing Radiation United Nations Scientific Committee on the Effects of Atomic Radiation, (2013).
[14] M.S. Pearce, J.A. Salotti, M.P. Little, K. McHugh, C. Lee, K.P. Kim, N.L. Howe, C.M. Ronckers, P. Rajaraman, A.W. Sir Craft, et al., Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study, Lancet 380 (9840) (2012) 499–505.
[15] G.M. Kendall, M.P. Little, R. Wakeford, K.J. Bunch, J.C. Miles, T.J. Vincent, J.R. Meara, M.F. Murphy, A record-based case-control study of natural background radiation and the incidence of childhood leukaemia and other cancers in Great Britain during 1980-2006, Leukemia 27 (1) (2013) 3–9.
[16] L. Krille, S. Dreger, R. Schindel, T. Albrecht, M. Asmussen, J. Barkhausen, J.D. Berthold, A. Chavan, C. Claussen, M. Forsting, et al., Risk of cancer incidence before the age of 15 years after exposure to ionising radiation from computed tomography: results from a German cohort study, Radiat. Environ. Biophys. 54 (1) (2015) 1–12.
[17] N. Journy, J.L. Rehel, H. Ducou Le Pointe, C. Lee, H. Brisse, J.F. Chateil, S. Caer-Lorho, D. Laurier, M.O. Bernier, Are the studies on cancer risk from CT scans biased by indication? Elements of answer from a large-scale cohort study in France, Br. J. Canc. 112 (1) (2015) 185–193.
[18] J.D. Mathews, A.V. Forsythe, Z. Brady, M.W. Butler, S.K. Goergen, G.B. Byrnes, G.G. Giles, A.B. Wallace, P.R. Anderson, T.A. Guiver, et al., Cancer risk in 680,000 people exposed to computed tomography scans in childhood or adolescence: data linkage study of 11 million Australians, Br. Med. J. 346 (2013) f2360.
[19] R. Doll, R. Peto, The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today, J. Natl. Cancer Inst. 66 (6) (1981) 1191–1308.
[20] S.H. Moolgavkar, D.J. Venzon, General relative risk regression models for epidemiologic studies, Am. J. Epidemiol. 126 (5) (1987) 949–961.
[21] K.J. Rothman, S. Greenland, Causation and causal inference in epidemiology, Am. J. Public Health 95 (Suppl 1) (2005) S144–S150.
[22] J.A. Sterne, G. Davey Smith, Sifting the evidence-what's wrong with significance tests? Br. Med. J. 322 (7280) (2001) 226–231.
[23] J.P. Ioannidis, Molecular bias, Eur. J. Epidemiol. 20 (9) (2005) 739–745.
[24] S. Goodman, S. Greenland, Assessing The Unreliability Of The Medical Literature: A Response To "Why Most Published Research Findings Are False, Johns Hopkins University, Dept Of Biostatistics Working Papers, 2007.
[25] L.A. Cox, P.F. Ricci, Empirical causation and biases in epidemiology: issues and solutions, Health Phys. 9 (1) (2002) 7.
[26] S.H. Mollgavkar, D.J. Venzon, Two-event models for carcinogenesis: incidence curves for childhood and adult tumors, Math. Biosci. 47 (1979) 22.
[27] I. Shuryak, L.B. Smilenov, N.J. Kleiman, D.J. Brenner, Potential reduction of contralateral second breast-cancer risks by prophylactic mammary irradiation: validation in a breast-cancer-prone mouse model, PloS One 8 (12) (2013) e85795.
[28] L.E. Feinendegen, Quantification of adaptive protection following low-dose irradiation, Health Phys. 110 (3) (2016) 276–280.
[29] M.H. Barcellos-Hoff, A.L. Brooks, Extracellular signaling through the microenvironment: a hypothesis relating carcinogenesis, bystander effects, and genomic instability, Radiat. Res. 156 (5 Pt 2) (2001) 618–627.
[30] K.N. Rithidech, Health benefits of exposure to low-dose radiation, Health Phys. 110 (3) (2016) 293–295.
[31] T.K. Hei, Response of biological systems to low doses of ionizing radiation, Health Phys. 110 (3) (2016) 281–282.
[32] K.T. Bogen, Linear-No-Threshold default assumptions for noncancer and non-genotoxic cancer risks: a mathematical and biological critique, Risk Anal. : an official publication of the Society for Risk Analysis 36 (3) (2016) 589–604.
[33] K.S. Crump, Bogen's critique of linear-No-threshold default assumptions, Risk Anal. : an official publication of the Society for Risk Analysis 37 (10) (2017) 1802–1807.
[34] NRC, Science and Decisions: Advancing Risk Assessment, National Research

Council, National Academy Press, Washington, DC, 2009.
[35] J. Hausman, Mismeasured variables in econometric analysis: problems from the right and problems from the left, J. Econ. Perspect. 15 (4) (2001) 11.
[36] P. Kennedy, A Guide to Econometrics, sixth ed., Wiley-Blackwell, 2008.
[37] W.K. Newey, Flexible simulated moment estimation of nonlinear errors-in-variables models, Rev. Econ. Stat. 83 (4) (2001) 12.
[38] Y. Hu, S.M. Schennach, Instrument variable treatment of nonclassical measurement error models, Econometrica 76 (1) (2008) 195.
[39] A. Spanos, Foundational Issues in Statistical Modeling: Statistical Model Specification and Validation Vol. 2 RRM, 2011.
[40] D.J. Brenner, R. Doll, D.T. Goodhead, E.J. Hall, C.E. Land, J.B. Little, J.H. Lubin, D.L. Preston, R.J. Preston, J.S. Puskin, et al., Cancer risks attributable to low doses of ionizing radiation: assessing what we really know, Proc. Natl. Acad. Sci. U. S. A 100 (24) (2003) 13761–13766.
[41] E.I. Azzam, N.W. Colangelo, J.D. Domogauer, N. Sharma, S.M. de Toledo, Is ionizing radiation harmful at any exposure? An echo that continues to vibrate, Health Phys. 110 (3) (2016) 249–251.
[42] J.F. Bithell, M.F. Murphy, C.A. Stiller, E. Toumpakari, T. Vincent, R. Wakeford, Leukaemia in young children in the vicinity of British nuclear power plants: a case-control study, Br. J. Canc. 109 (11) (2013) 2880–2885.
[43] D.J. Brenner, R.K. Sachs, Estimating radiation-induced cancer risks at very low doses: rationale for using a linear no-threshold approach, Radiat. Environ. Biophys. 44 (4) (2006) 253–256.
[44] NCRP, Health Effects of Low Doses of Radiation: Perspectives on Integrating Radiation Biology and Epidemiology, (2015).
[45] J.S. Puskin, Perspective on the use of LNT for radiation protection and risk assessment by the U.S. Environmental Protection Agency, Dose-response : a Publication of International Hormesis Society 7 (4) (2009) 284–291.
[46] NRC, Health Effects of Radon and Other Internally Deposited Alpha-Emitters (BEIR IV), National Academy Press, Washington, DC, 1988.
[47] NRC, Health Effects of Exposure to Low Levels of Ionzing Radiation (BEIR V), National Academy Press, Washington, DC, 1990.
[48] NRC, Health Effects of Exposure to Radon (BEIR VI), National Academy Press, Washington, DC, 1999.
[49] NIH, Report of the NCI-CDC Working Group to Revise the 1985 NIH Radioepidemiological Tables, In NIH Publication No. 03-5387, Bethesda, MD, 2003.
[50] P. Armitage, R. Doll, The age distribution of cancer and a multi-stage theory of carcinogenesis, Br. J. Canc. 8 (1954) 1–12.
[51] D.C. Doll, R.B. Weiss, S. Shah, Lymphoma of the prostate presenting as benign prostatic hypertrophy, South. Med. J. 71 (1978) 1170–1171.
[52] A.G. Knudson, A personal sixty-year tour of genetics and medicine. Annual review of genomics and human genetics, Annu. Rev. Genom. Hum. Genet. 6 (2005) 1–14.
[53] H. Norppa, S. Bonassi, I.L. Hansteen, L. Hagmar, U. Stromberg, P. Rossner, P. Boffetta, C. Lindholm, S. Gundy, J. Lazutka, et al., Chromosomal aberrations and SCEs as biomarkers of cancer risk, Mutat. Res. 600 (2006) 37–45.
[54] S.H. Moolgavkar, R.G. Stevens, J.A. Lee, Effect of age on incidence of breast cancer in females, J. Natl. Cancer Inst. 62 (1979) 493–501.
[55] M. Greaves, Darwinian medicine: a case for cancer, Nat. Rev. Canc. 7 (2007) 213–221.
[56] M.A. Nowak, F. Michor, Y. Iwasa, The linear process of somatic evolution, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 14966–14969.
[57] E.J. Baker, J.J. Jay, V.M. Philip, Y. Zhang, Z. Li, R. Kirova, M.A. Langston, E.J. Chesler, Ontological discovery environment: a system for integrating gene-phenotype associations, Genomics 94 (2009) 377–387.
[58] J.W. Tukey, The Collected Works of John W. Tukey VIII. Multiple Comparisons: 1948–1983, Taylor & Francis, 1994.
[59] A.J. Hayter, J.C. Hsu, On the relationship between stepwise decision procedures and confidence sets, J. Am. Stat. Assoc. 89 (1994).
[60] J.C. Greene, Advances in Mixex-Method Evaluation: The Challenges and Benefits of Integrating Diverse Paradigms, Wiley, 1997.

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Economic benefit-cost implications of the LNT model

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1. Introduction

As documented in this Special Issue, the linear no threshold (LNT) dose response model as a default model is scientifically invalid for both radiation and most chemicals. Consequently, there is no logical rationale to assume that the LNT model should be used to estimate health or safety benefits within benefit-cost analysis. Because thresholds are likely to exist for both radiation and chemicals, assuming that LNT is valid for economic analyses will lead to policy decisions with unnecessary costs imposed on society. From an economic perspective, a *policy threshold* is reached when the costs of decreasing exposures exceed the benefits. This paper investigates the use of LNT and policy thresholds using two examples to illustrate them, radon and formaldehyde.

EPA's present LNT-driven program to mitigate indoor air radon to prevent lung cancer from homes and buildings costs billions of dollars each year while the benefits of the program are, at best, negligible. EPA's program to reduce exposure from formaldehyde in composite wood products costs about $60 million each year, again, with negligible (or zero) benefits. Both programs demonstrate that the economic threshold for decreasing exposure has, for the most part, already been attained and would have been correctly identified if a threshold model had been employed. Thus, use of a threshold model, instead of an LNT model, would have resulted in a different policy.

Controversy over the use of LNT for risk assessment purposes goes back over 70 years to 1946 when Ernst Caspari reported a threshold response to radiation, based on the dose rate for gamma-ray-induced mutations in fruit flies [1]. Prior to that, ionizing radiation-induced mutations were assumed to be linear, down to zero (i.e., a single "hit" could induce cancer), with respect to the dose. Caspari's findings challenged the model originally created by Herman Muller.

In fact, Muller believed he had induced mutations by dosing fruit flies with X-rays when, in fact, he made "large gene deletions and other gross chromosomal aberrations [2]." After Mullers mistakes, Caspari's findings set off a temporary alarm amongst the radiation genetics community such that it prompted one radiation researcher to ask (about the LNT), "What can we do to save the (one) hit model? [1]" Their worry was misplaced; Muller's views eventually won the day.

But Ed Calabrese reports that "the LNT dose-response model, which drives cancer risk assessment, was based on flawed science, on ideological biases by leading radiation geneticists, on scientific misconduct by an NAS Genetics Panel during the atomic radiation scares of the 1950s, and on a 40 year mistaken assumption by yet another NAS Committee [3]."

The controversy is important for multiple reasons. Scientifically, as extensively discussed in this Special Issue, the LNT makes no sense biologically. Second, from a risk perspective, chemicals and radiation are regulated to very low levels and those regulations often replace a very low risk with a higher risk from a substitute product or activity. This is called a risk/risk trade-off. Finally, use of the LNT inappropriately can lead to the imposition of unnecessary, and often very large, costs.

It is no longer possible to ignore the costs of regulation. Today, nearly 300,000 federal workers (up from 57,000 in 1960 [4] put out 3–4000 regulations every year that have resulted in over 1 million restrictions (individual requirements) in the *Code of Federal Regulations* [5]. The cost of these regulations to the U.S. economy, although difficult to estimate, could be as high as $2 trillion each year [6]; nearly 11% of the U.S. GDP.

Inappropriate use of the LNT can lead to spending too much on regulations and, because it results in overestimation of risks, will in turn cause benefits to be overestimated. Most benefit analyses currently use the results of risk assessments as the starting point. To be useful with estimates of cost, risk assessments need to estimate actual risks and to factor in the probabilistic information when possible to properly characterize the expected and net risks [7].

Beyond costs and benefits, there are other problems with using the LNT when it is not appropriate. In a staff report, EPA declares that it seeks to adequately protect public and environmental health by preferring an approach that does not underestimate risk in the face of uncertainty and variability. In other words, EPA seeks to adequately protect public and environmental health by *ensuring that risk is not likely to be underestimated"* [emphasis in original] [8]. To ensure that they do not underestimate risk, EPA staff routinely employ conservative defaults and assumptions that result in substantial overestimates of risk. Employing the LNT when there is a threshold is also conservative (precautionary). But by doing so, they have "effectively usurped risk management since managers (are) often never made aware of

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uncertainties or the potential impact of these uncertainties on the results and (are) essentially forced to make decisions that began with an assumed overestimate of the risk [7]."

2. Thresholds

Studies have shown that the same mechanisms that work at high doses for cancer causing substances, do not work at low doses because "we have evolved molecular systems that continuously monitor and repair DNA." [9] Others have argued, however, that because there is an underlying rate of spontaneous cancers, that induced cancers (from toxic substances and radiation) add to the probability of getting cancer. However, a recent evaluation of the mechanisms by which cancer is produced from spontaneous and induced cancers shows that they have completely different mechanisms. Therefore, they cannot be additive [10].

All chemicals will have a threshold because assaults to biological systems arise continuously and from everywhere - plants and animals we eat, environmental stressors, sunlight and other background radiation, oxygen, and microorganisms such as pathogens and viruses. Whether there are potentially negative effects from a stressor depends on dose, medical status and other factors.

3. Benefit-cost thresholds

Policy thresholds underlie the decision thresholds used by risk managers to determine when exposure is low enough to stop regulating. The key economics concept behind benefit-cost thresholds is "opportunity cost." Opportunity costs arise because decisions must be made among regulatory options. The opportunity cost of choosing one course of action is not choosing a different option. For example, if a regulation requires managers to switch from creating a new product to complying with a regulation, the opportunity cost is forcing them to spend their time on the regulation rather than their preferred option, creating the new product. Social opportunity costs include preventing people from using their existing resources on current activities as well as preventing them from buying something new. For example, if we require money to be spent on a federal regulation to reduce the risk of formaldehyde, the opportunity cost might be that worker safety could be improved elsewhere.

Policy decisions that employ benefit-cost thresholds could be, for example, how low a level to set for limiting exposure to a harmful chemical, whether to force a manufacturer to buy a certain kind of equipment to make a product or plant safer, how clean fill dirt must be in a Superfund site, how long to give manufacturers to comply with a regulation, or whom should be covered by a regulation or law. Each policy decision causes organizations, and possibly consumers, to change behaviors that, in turn, affects the type and magnitude of risks (benefits) they face. Costs are the forced changes in behaviors (e.g., new management or labor requirements, new capital) that move people away from their preferred option.

In a health and safety benefit-cost analysis, benefits are calculated as the (expected) reduced risk of morbidity or mortality for a population multiplied by the value people place on those reduced risks. People value each unit of risk reduced and those values can be estimated. For example, buying a car with more costly safety features implies a trade-off for other features, such as a larger engine.

The opportunity costs of choosing a very low level for limiting exposure to a chemical or radiation are that the resources might have been spent reducing exposure elsewhere. When the additional costs of reducing exposure to a lower level (i.e., what has been given up) is more than the additional benefits of that reduction, i.e., the marginal costs are greater than the marginal benefits, then the economic threshold has been reached. This explains why the results of a benefit-cost analysis are said to generate an "efficient" choice. An efficient choice means that every dollar spent obtains the maximum possible benefit, i.e., the biggest bang for the buck. Morrall has shown how opportunity costs can be illustrated by the cost per life saved for various risk-reducing regulatory actions [11].

Crump describes an economic threshold as one that is "a societal decision rather than a purely scientific one [12]." Although not always the case, as regulation attempts to decrease exposure to a hazard, each reduction in exposure costs more than the previous reduction. Another way to say this is, each dollar spent will reduce exposure less than the previous dollar.[1]

An example of this principle comes from Superfund. When cleaning up a Superfund site, the deeper the ground that must be cleaned, the more it costs to go an additional yard deeper. Supreme Court Justice Stephen Breyer explained why the marginal costs and marginal benefits of cleaning Superfund sites matter in *Breaking the Vicious Circle.*

The first comes from a case in my own court, *United States v. Ottati & Goss,* arising out of a ten-year effort to force cleanup of a toxic waste dump in southern New Hampshire. The site was mostly cleaned up. All but one of the private parties had settled. The remaining private party litigated the cost of cleaning up the last little bit, a cost of about $9.3 million to remove a small amount of highly diluted PCBs and 'volatile organic compounds' (benzene and gasoline components) by incinerating the dirt. How much extra safety did this $9.3 million buy? The forty-thousand-page record of this ten-year effort indicated (and all parties seemed to agree) that, without the extra expenditure, the waste dump was clean enough for children playing on the site to eat small amounts of dirt daily for 70 days each year without significant harm. Burning the soil would have made it clean enough for the children to eat small amounts daily for 245 days per year without significant harm. But there were no dirt-eating children playing in the area, for it was a swamp. Nor were dirt-eating children likely to appear there, for future building seemed unlikely. The parties also agreed that at least half of the volatile organic chemicals would likely evaporate by the year 2000. To spend $9.3 million to protect non-existent dirt-eating children is what I mean by the problem of "the last 10% [13]."

The opportunity cost of the resources spent on that last 10% might have been better spent on reducing risks of unintentional injuries to children (the leading cause of death for children aged 1–4) [14].

The marginal costs and marginal benefits of reducing exposure are illustrated in Fig. 1.

In Fig. 1, point A is where marginal costs of reducing exposure equal marginal benefits. Any decision to reduce exposure below point A (to the right) has marginal costs exceeding marginal benefits. Point B is the effect threshold, where any reduced exposure beyond that has only costs, no benefits.

When using an LNT model and ignoring a threshold, perhaps to be *protective* as EPA attempts to be, false benefits will be attributed to a reduction in exposure. Using the LNT inappropriately moves the benefit-cost threshold to the right of point A where, in actuality, marginal costs exceed marginal benefits. What's worse, this fact is obscured. As radiation and most chemicals have a toxicological threshold, inappropriate use of the LNT as a default is not only inaccurate but costly. The two examples below demonstrate how ignoring thresholds generates costs that vastly exceed benefits.

4. Radon

Radon-222 is a gas, a so-called "daughter" of radium 226 that is ubiquitous in the earth's crust and can be found in many homes and commercial buildings. EPA cites the Surgeon General's Health Advisory that "Indoor radon is the second leading cause of lung cancer in the United States [15] …"

Some states and localities have produced their own radon laws

[1] See, for example, Miller, Wilhelmine et al., National Academy of Sciences, "Valuing Heath for Regulatory Cost-Effectiveness Analysis," 2006, p. 267.

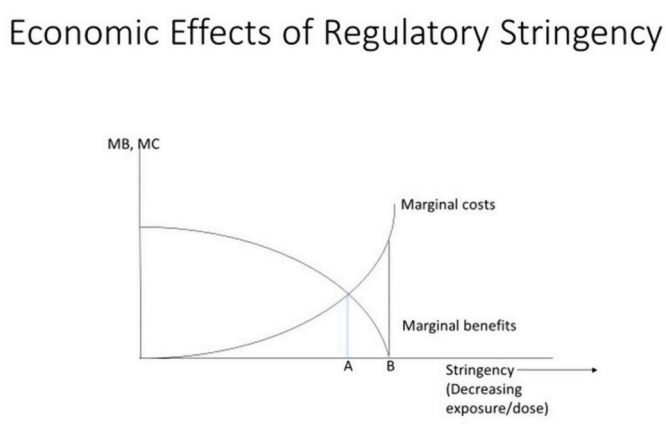

Fig. 1. Economic effects of regulatory stringency.

including 37 states with their own requirement for real estate transaction disclosure [16]; 11 states requiring radon resistant new construction; 2 states requiring testing in day care centers; and 18 states having radon mitigation laws [16].

At the federal level, EPA has a national program for testing and remediating radon for both homes and commercials buildings, particularly if they are present at levels at or above 4 pCi/L.[2] EPA based their 2003 reassessment of the risk of lung cancer from radon on the National Academy of Science BEIR VI (Biological Effects of Ionizing Radiation) report that used the LNT model [17]. EPA has data showing that 6% of all homes have "elevated levels" of indoor radon (above 4 pCi/L) [18]. CDC estimates that 7 million homes have high radon levels [19]. Because radon levels may vary considerably between counties and even adjacent homes, EPA warns that, "All homes should be tested, regardless of (radon) zone designation [20]." Sale of homes is typically contingent on the seller providing certification of a low radon home (or building) and proof of such shows up in most real estate contracts.

It is estimated that there are approximately 600,000 homes sold in the U.S. each year [21]. With an average testing cost of about $500 per home [22], the total cost to test all homes is about $30 million per year. If a seller must provide remediation for radon, EPA estimates that the average cost is between $800 and $2500 per house ($1600 average) [15]. Assuming that only those homes above 4 pCi/L are remediated, then 36,000 homes are remediated each year at a cost of $58 million annually. Together, testing and remediation are estimated to cost $89 million annually.[3]

In 2012, there were 5.6 million new commercial buildings in the U.S. comprising 87 billion square feet [23]. Assuming the same percentage of buildings as homes need radon remediation (6%), 336,000 buildings with an average of 15,536 square feet would be remediated each year. However, as radon cannot be tested until a building is built, it may be that in the 26 states that have high radon levels (average over 4 pCi/L), new buildings in these states may be built to eliminate radon during construction [24].

The average costs for radon remediation including engineering (vapor barriers), installation, first-year maintenance, and administrative costs for a new commercial building is $67,000, with an additional $7500 beginning in the second year in annual maintenance costs [25]. If only 6% of new buildings are remediated (the same percentage as homes above 4 pCi/L), the costs would be $23 billion per year. If half of all new buildings are remediated (in high radon states), the cost would be $189 billion per year. These figures only include new buildings, not existing buildings that test high and must be remediated.

[2] There are many ways to measure radiation, picocuries per liter. pCi/L, is a measure of the rate of radioactive decay of radon.

[3] EPA recommends remediation even down to 2 pCi/L.

Despite the Surgeon General's finding, on-going benefits of this program are suspect due to the high degree of uncertainty in the attributable levels of lung cancer related to radon. In fact, the threshold level for radon is most likely much higher than the EPA "action level." Higher minimum levels for carcinogenic effects have been reported, 8.1 pCi/L [26] and 14.7 pCi/L [27]. In addition, a recent paper that reanalyzed 32 case-control and two ecological studies concerning radon's effect on lung cancer risk concluded that exposure to radon concentrations below about 27 pCi/L (1,000 /bq/m^3) were not associated with any "statistically significant increase in lung cancer incidence [28]." Exactly where the threshold lies is uncertain, but it is likely to be somewhere between 8 and 27 pCi/L, or more than twice EPA's action level of 4 pCi/L. In fact, 4 pCi/L appears to be a level that is protective, not harmful [17].

Maps of average radon levels in "high" radon states (averages above 4 pCi/L) show that only two states have averages above the likely lowest threshold, 8 pCi/L [29]. South Dakota has an average of 9.6 pCi/L and Pennsylvania has an average of 8.6 pCi/L [29]. Both states have average levels well below 27 pCi/L, although, there is no available data as to how many individual homes and buildings exceed that concentration.

Fig. 2 indicates that the majority of lung cancer is caused by smoking, not radon.

Based on Fig. 2, a more efficient (cost effective) way to reduce lung cancer would be to address smoking directly. For example, to reduce the number of smokers, the Centers for Disease Control created a program (Tips from Former Smokers) that ran for 3 months in 2012. CDC estimated the program was likely to cause 100,000 people to quit smoking [31]. The campaign cost $54 million or about $540 per quitter, assuming the number of quitters' estimate proved to be correct. Given that most of the homes and buildings have radon levels far below the thresholds that have been discussed in this paper, it's likely that the number of cases of lung cancer reduced by radon mitigation are far less than the BEIR report shows suggesting that trying to reduce cases of lung cancer by mitigating radon results in expenditures of billions of dollars per case avoided. This means that the expenditures (costs) are likely to vastly exceed the benefits.

5. Formaldehyde

EPA recently finalized a regulation governing formaldehyde emissions for composite wood products (Formaldehyde Standards for Composite Wood Products Act, or Title VI of TSCA, 15 U S C. 2697) [32]. The primary health effect quantified by EPA in this regulation was for nasopharyngeal cancer (NPC). EPA's risk estimates were derived from the International Agency for Research on Cancer [33,34], the National Toxicology Program [35] and the US EPA [36](2010a), which concluded that formaldehyde is a known human carcinogen for NPC. However, that conclusion was based almost exclusively on the results of a single study conducted by the National Cancer Institute (NCI), which reported 9 deaths from NPC among more than 25,000 workers exposed [37]. Notably, five deaths came from one plant (Plant 1), while the remaining 4 were randomly distributed in the other 9 plants.

EPA presented an economic analysis (RIA) for its composite wood products regulation the LNT model-based IRIS Inhalation Unit Risk factor of 1.3×10^{-5} cancer cases per μg/m3 of formaldehyde (an upper bound) [38]. Using a unit risk factor implies that any reduction in risk would be the same for every reduction in exposure, and that there is no threshold for its cancer potency (i.e., the LNT model).

EPA estimated that reducing formaldehyde exposure to 1 ppm from current levels would, using the IRIS unit risk factor, result in 26–65 cases of NPC avoided per year (with annual benefits of between $19.3 to $47.6 million per year) [38]. However, the expected cases avoided are greater than the number of NPC cases from all exposure sources by a factor of more than 20 [39]. In fact, the World Health Organization concluded that there is no evidence of NPC caused by exposure to

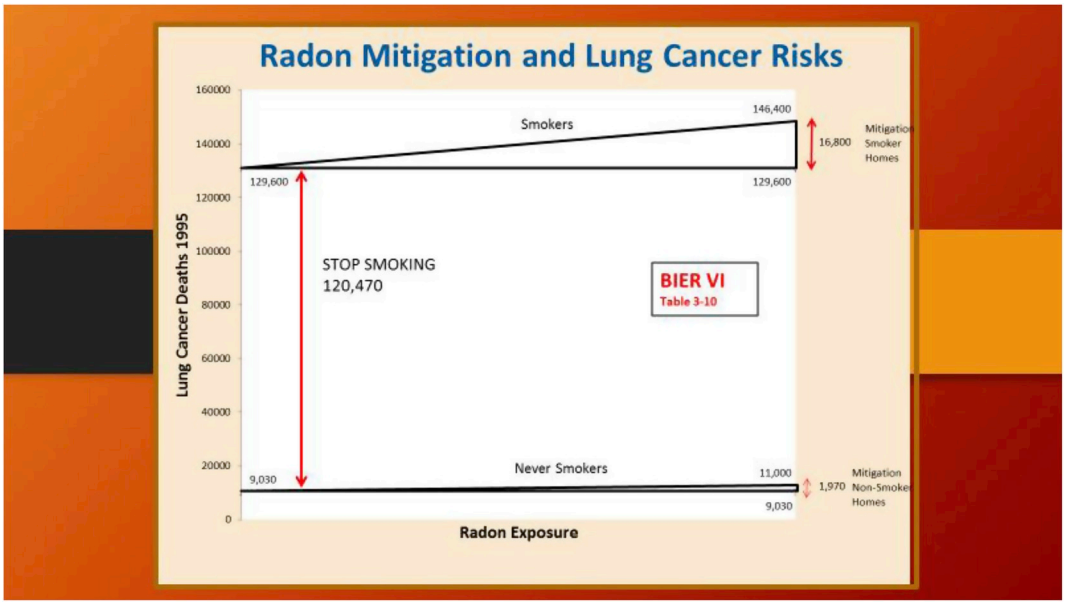

Fig. 2. Radon Mitigation and lung cancer risks [30].

formaldehyde at mean concentrations below 1.25 mg/m^3 [40].

Following the IRIS assessment (that produced formaldehyde's inhalation unit risk factor), McGregor determined that the mode-of-action elements (i.e., cytotoxicity, cell proliferation, and DNA effects) for formaldehyde-induced nasal tumors are not linear but, in fact, highly non-linear and do not occur unless a threshold dose (6 ppm) has been exceeded (well above EPA's action level of 1 ppm) [41]. In addition, Conolly found that upper respiratory tract cancers most likely had a *de minimis* level of 10^{-6} or less at relevant workplace exposure levels [42].

Careful investigation of the previous employment history of Plant 1 workers who died from NPC determined that four of the five NPC cases had worked previously in silver-smithing occupations involving substantial exposures to potential known risk factors for upper respiratory system cancers, including sulfuric acid mists and metal dusts [43]. In fact, in an updated re-analysis of the mortality risk from NPC in the NCI formaldehyde worker cohort Marsh [44] (2016) concluded that there was "little or no evidence to support NCI's suggestion of a persistent association between FA exposure and mortality from NPC. NCI's suggestion continues to be driven heavily by anomalous findings in one study plant (Plant 1)."

In another study of more than 14,000 British chemical workers with elevated formaldehyde exposures (including some 4000 workers with exposures >2 ppm), there was no evidence of elevated NPC. The authors of this study (which involved formaldehyde exposures in excess of the NCI cohort) concluded that the evidence for formaldehyde carcinogenicity in humans was unconvincing [45]. In a study by the National Institute of Occupational Safety and Health (NIOSH) of more than 11,000 garment workers occupationally exposed to formaldehyde, no cases of NPC were observed [46]. Finally, a study from NCI conducted by Hauptmann [47] of formaldehyde-exposed embalmers reported no excess of NPC in the cohort.

Notably, the excess of NPC cases in the NCI cohort (Plant 1), quite reasonably now attributable to other exposures, appear unlikely to be related to formaldehyde. It is difficult to envision a scenario in which the 6 cases of NPC in the approximately 7000 workers in Plant 1 were due to formaldehyde when none occurred among 25,000 plus occupationally exposed workers reported in the other studies.

Interestingly, EPA's use of the IRIS unit risk factor implies that there is a 1 in one million risk of NPC at a formaldehyde exposure concentration of 0.08 parts per *billion.* This level is more than 50 times lower than the median concentration people (and all animals) exhale in each breath (4.3 ppb) resulting from normal endogenous metabolic processes [48]. In fact, Golden concluded that "a formaldehyde concentration of 0.1 ppm would be *protective* for leukemia or cancer at any other site within the body [28,49]."

Since the IARC (2006) decision concluding that formaldehyde is a known human carcinogen based on NPC, several comprehensive quantitative evaluations of the epidemiological literature have carefully documented that the weight of human evidence does not support a causal association between formaldehyde exposure and NPC [50,51]. EPA's reliance on the single NCI study now seems to be entirely unwarranted and the benefits of this rule are likely to approach zero.

The costs of EPA's composite wood products rule included "changes to production process and raw materials that are needed to meet the emissions standards, as well as the costs of the testing, third-party certification, rule familiarization, recordkeeping, labeling, and chain of custody activities required by the rule." were estimated to be from $38 million to $83 million per year [42]. Golden has concluded, "Despite numerous epidemiology studies that have raised a specter of formaldehyde-induced NPC and leukemia, both endpoints now appear more likely to be false positives, as these findings are inconsistent with an ever-increasing body of data demonstrating that such effects simply cannot occur under any real-world exposure scenario [49]." If Golden's conclusion is correct, the likely benefits of the composite wood products rule are negligible, if not zero, meaning that the net costs of the rule are $38 to $83 million per year.

5.1. Leukemia and formaldehyde

EPA also considered the risk of leukemia and formaldehyde. However, in their Regulatory Impact Analysis, they concluded, "EPA did not have sufficient information to derive a concentration-response function for myeloid leukemia and thus could not estimate the number of cases that would be avoided by reducing formaldehyde exposure [32]." While EPA did not quantify the effects of formaldehyde on leukemia in their most recent regulation, the International Agency for Research on Cancer (IARC 2009) has concluded that there is "sufficient evidence" to link formaldehyde with leukemia [33].

While a number of chemicals (e.g., benzene and some anti-cancer drugs) have been associated with leukemia, all share the ability, following inhalation, to enter the blood with subsequent transport to the bone marrow where leukemia develops. Given the prodigious

metabolism and detoxification of formaldehyde in the upper respiratory tract, no inhaled exogenous formaldehyde, even at high concentrations, can be detected in the blood to increase the concentrations already present naturally. Since leukemia is far more prevalent that NPC, it is feasible that EPA, will at some point rely on LNT to reduce exposure limits to mitigate potential risks of this endpoint as well.

6. Conclusion

Risk management decisions can be based on different factors, such as the need to protect a highly exposed or sensitive group of people or a legal requirement. Because different legal requirements and values affect risk management decision-making, different thresholds may need to be considered in benefit-cost determinations. For policy decisions based on benefit-cost analysis, using the LNT model when it is biologically inappropriate causes benefits to be over-estimated and results in costs more than what they would be worth to consumers, who ultimately pay for regulations. Use of the LNT for risk management may be viewed as “conservative” because it overestimates risk to ensure public health protection. However, spending scarce resources to prevent some risks means that we may not be addressing others. In other words, the more regulators try to lower exposure to chemicals or radiation, particularly past their toxicity thresholds, the more likely they are to get the policy wrong. As extensively addressed in the other papers in this Special Issue, because LNT is an invalid dose-response descriptor for potentially carcinogenic effects from both chemicals and radiation, it should not be used in economic analyses.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.cbi.2019.01.028.

References

[1] Edward J. Calabrese, Flaws in the LNT single-hit model for cancer risk: an historical assessment, Environ. Res. (2017) 773–788.
[2] Calabrese, J. Edward, The EPA Cancer Risk Assessment Default Model: Moving Away from the LNT. et. al. s.l, Sage Journals: Dose-Response, 2018.
[3] J. Edward, PhD. Calabrese, Oversight of the Environmental Protection Agency's Implementation of Sound and Transparent Science in Regulation, (2018) Washington DC, Oct 3.
[4] S. Dudley, M. Warren, An analysis of the U.S. Budget for fiscal years 1960 to 2018, p.6. Regulators Budget Report (2018) [Online] https://regulatorystudies.columbian.gwu.edu/sites/g/files/zaxdzs1866/f/downloads/2018_Regulators_Budget-2017-07-18.pdf.
[5] Mercatus Center at GMU. The accumulation of regulatory restrictions across presidential administrations. QuantGov. [Online] https://quantgov.org/regdata/the-accumulation-of-regulatory-restrictions-across-presidential-administrations/.
[6] National Association of Manufacturers, The Cost of Federal Regulation to the U.S. Economy, Manufacturing and Small Business, (2012) NAM. [Online] http://www.nam.org/Data-and-Reports/Cost-of-Federal-Regulations/Federal-Regulation-Executive-Summary.pdf.
[7] R. Williams, K. Thompson, Integrated Analysis: Combining Risk and Economic Assessments while Preserving the Separation of Powers, Risk Analysis, 2004.
[8] Office of Science Advisor, EPA, Examination of EPA Risk Assessment and Principles and Practices, EPA, Washington DC, 2004 (Staff report).
[9] Royal Swedish Academy of Sciences, Mechanaistic Studies of DNA Repair, The Royal Swedish Academy of Sciences, Stockholm, 2015.
[10] The additive to background assumption in cancer risk assessment: a reappraisal, Calabrese, Edward J. (2018) 175–204 Environmental Researhc.
[11] Saving Lives: A review of the record, Morrall, F. John, J. Risk Uncertain. (2003) 221–237.
[12] Use of threshold and mode of action in risk assessment, K.S. Crump, Crit. Rev. Toxicol. (2011) 637–650.
[13] Stephen Breyer, Breaking the Vicious Circle, Harvard College, Boston, 1993.
[14] CDC. FastStats Homepage, National Center for Health Statistics, (2017) [Online] May 3 https://www.cdc.gov/nchs/fastats/child-health.htm.
[15] EPA, Consumers Guide to Radon Reduction: How to Fix Your Home, EPA, Washington DC, 2010 (Consumer Guidance).
[16] E. Geltman, The Policy Surveillance Portal, Law Atlas (2016) 1–1. [Online] Dec 1 http://lawatlas.org/datasets/state-radon-laws , Accessed date: 31 January 2019.
[17] Bobby R. Scott, Residential radon appears to prevent lung cancer, Dose-Response (2011) 333–464.
[18] EPA, Home Buyer's and Seller's Guide to Radon, EPA, Washington DC, 2018 (Consumer Guidance).
[19] CDC. Policies and practices for cancer prevention. Reducing Household Radon, p. 1.. [Online] [Cited: September 6, 2018.] https://www.cdc.gov/cancer/dcpc/pdf/HouseholdRadon.pdf.
[20] EPA. Find information about local radon zones and state contact information. [Online] https://www.epa.gov/radon/find-information-about-local-radon-zones-and-state-contact-information.
[21] Statista. Number of New Houses Sold in the united States from 1995 to 2017 (In Thousands). The Statistics Portal. ([Online]).
[22] Test for radon gas. Home Advisor, p. 1. [Online] https://www.homeadvisor.com/cost/environmental-safety/test-or-remove-radon-gas/.
[23] US Energy Information Administration, A Look at the U.S. Commercial Building Stock: Results from EIA's 2012 Commercial Buildings Energy Consumption Survey (CBECS, US EIA, Washington, 2015.
[24] Radon.com. [Online] http://www.radon.com/maps.
[25] Doug Kilmer, et al., Vapor Intrusion Mitigation: Let Me Count the Ways. s.L, TRC White Paper, 2015.
[26] Epidemiological Evidence for Possible Radiation Hormesis from Radon Exposure: A Case-Control Study Conducted in Worcester, Thompson, RE., MA, 2011, pp. 59–75 Dose Response.
[27] BL. s.l. Cohen, Test of the linear-No threshold theory of radiation carcinogenesis for inhaled radon decay producdts, Health Phys. (1995) 157–174 Health Physics.
[28] L. Dobrzynski, et al., Meta-analysis of thirty-two case–control and two ecological radon studies of lung cancer, J. Radiat. Res. (2017) 1–15, https://doi.org/10.1093/jrr/rrx061.
[29] Air Radon, Interactive radon map, [Online] 6, Air Radon.com (2018) September https://www.radon.com/maps/.
[30] BEIR VI Radon: The rest of the story, A.L. Brooks, D.G. Hoel, s.n Cancun, Radiation Research Society (2017) 12.
[31] CDC. More than 100,000 Americans quit smoking due to national media campaign. CDC newsroom. [Online] Sept 9, 2013, p. 1. https://www.cdc.gov/media/releases/2013/p0909-tips-campaign-results.html.
[32] EPA, Economic and Policy Analysis Branch, Formaldehyde Emission Standards for Composite Wood Products, regulations.gov. [Online] Dec 12, 2016. [Cited: August 16, 2018.] https://www.regulations.gov/document?D=EPA-HQ-OPPT-2016-0461-0001.
[33] Formaldehyde, mechanistic and oother relevant data, Monograph No. 09, Lyone : IARC 100F International Agency for Research on Cancer, 2009 WHO.
[34] Flormaldehyde, 2-Butoyethanol and 1-Tert-Butoxypropan-2-Ol. . IARC, IARC monographs on the Evaluatio n of Carcinogenic Risks to Humans, Lyon, 2006.
[35] National Toxicology Program, Formaldehy Expeert Panel Report Part B. Recommendation for Listing Status for Formaldehyde and ?Scientific Justification for the Recommendation, NTP, Research Triangle Park, 2009.
[36] U.S. EPA, Formaldehyde-inhalation assessment (CAS No 50-00-0), Support of Summary Information on the Integrated Risk Information Sysstem (IRIS, U.S. Environmental Protection Agency, Washington D.C., 2010.
[37] M. Hauptman, et al., Mortality from solid cancers among workers in formaldehyde industries, Am. J. Epidemiol. 159 (2004).
[38] Economic and Policy Analysis Branch, Economic Analysis of the Formaldehyde Standards. s.L. : EPA, RIA, July, 2016.
[39] National Cancer Institute. SEER Cancer Statistics Review 1975-2010 Tables 20.8 and 20.9.
[40] World Health Organization, WHO Guidelines Fo rIndoor Air Quality: Selected Pollutants, (2010).
[41] D. McGregor, et al., Formaldehyde and glutaraldehyde and nasal cytotoxicity: case study within the context of the 2006 IPCS Human Framework for the Analysis of a cancer mode of action for humans. s.l, Criticial Reviews in Toxicology 36 (10) (2006) 353–354, https://doi.org/10.1080/10408440600977669.
[42] R.B. Conolly, et al., et. al., Human respiratory tract cancer risks of inhaled formaldehyde: dose-response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset, Toxicology Science 82 (1) (2004) 279–296.
[43] G.M. Marsh, et al., Work in the metal industry and nasopharyngeal cancer mortality among formaldehyde-exposed workers, Regul. Toxicol. Pharmacol. 48 (2007).
[44] G.M. Marsh, et al., et.al., An updated re-analysis of the mortality risk from nasopharyngeal cancer in the National Cancer Institute formaldehyde worker cohort study, J. Occup. Med. Toxicol. 11 (8) (2016), https://doi.org/10.1186/s12995-016-0097-6.
[45] D. Coggon, et al., Extended follow-up of a cohort of British chemical workers exposed to formaldehyde, J. Natl. Cancer Inst. (2003) 1608–1615.
[46] L.E. Pinkerton, Mortality among a cohort of garment workers exposed to formaldehyde: an update, Occup. Environ. Med. (2004) 193–200.
[47] M. Hauptman, Mortality from lymphohematopoietic malignancies and brain cancer among embalmers exposed to formaldehyde, J. Natl. Cancer Inst. (2009) 1696–1708.
[48] B. Moser, et al., et. al., Mass spectrometric profile of exhaled breathfield study by PTR-MS. s.l. Respiratory Physiological Neurobiology 145 (2–3) (2005) 295–300, https://doi.org/10.1016/j.resp.2004.02.002.
[49] Identifying an indoor air exposure limit for formaldehyde considering both irritation and cancer hazards. Golden, Robert. 1, s.l. Crit. Rev. Toxicol. 1 (8) (2011) 672–721, https://doi.org/10.3109/10408444.
[50] C. Borsetti, et al., Formaldehyde and cancer risk: a quantitative review of cohort studies through 2006, Ann. Oncol. (2008) 29–43.
[51] S. Duhayon, et al., Carcinogenic potential of formaldehyde in occupational settings: a critical assessment and possible impact on occupational exposure levels, Int. Arch. Occup. Environ. Health (2008) 695–710.