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A Mathematical Modeling Approach to Characterize Hormesis, Caloric Restriction and Toxicity in Mortality Data from Toxicity Studies

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Abstract: The mortality of a population reflects the combined effects of natural aging with environmental insults (e.g. toxicant exposure, caloric restriction, over feeding, infections, etc.). No single endpoint, such as carcinogenesis, completely describes toxicant-induced effects in laboratory animals. Mathematical models of mortality and time-dependent exposure to toxicants can yield indices of net injury from all causes. With careful manipulation of diet in control groups, the separate and combined effects of hormesis, caloric restriction and toxicity on mortality can be delineated. In particular, age-specific mortality rate analyses can characterize a) the temporal pattern of net injury from acute, short term and chronic environmental exposure, b) late-life effects, c) effects of fractional exposures and d) relative biological effectiveness. Changes in the design of toxicity studies and analyses of mortality data are suggested.

Key words: Hormesis, caloric restriction, mortality, chronic toxicity

INTRODUCTION

Hormesis and caloric restriction have been considered as related phenomena in laboratory rodents (see Human and Experimental Toxicology vol. 19 no 6, 2000).[1] Laboratory rodents are typically inbred animals given ad libitum access to food - a condition that does not mimic the wild animal from which it was originally bred. These animals are actually over fed in captivity.[2,3] Caloric restriction of laboratory rodents reduces mortality of these animals. One common effect of chronic exposure of laboratory rodents to toxic substances is that the animals eat less.[4] The resulting reduction in food intake may mask any increase in mortality from the exposure to the toxic substance.

Hormesis is a phenomenon that is well conserved across species.[5] Relatively little attention, however, has been given to modeling mortality data from chronic toxicity studies to characterize the separate effects of hormesis and caloric restriction. This paper examines the separate effects of hormesis, caloric restriction and toxicity on mortality data and proposes changes in the design and analyses of toxicity studies to determine the dose-mortality response properties of substances that elicit hormesis.

MATERIALS AND METHODS

The hazard function (instantaneous age-specific mortality rate) multiplied by dx, is the probability of death during the interval x to x + dx (where dx is infinitesimal). This assumes the individual has survived to the beginning of the interval. The mathematical definition of the hazard function is[6]

$$h(x) = \frac{-d[\ln S(x)]}{dx} = -\frac{1}{N_x}\frac{dN_x}{dx}, \qquad (1)$$

where, h(x) is the hazard function at age x, (age-specific mortality rate), S(x) is the fraction of the population surviving at age x and N_x is the number of individuals surviving at age x. An age-specific mortality rate of 0.12 $week^{-1}$ at age 50 weeks means that the probability of an individual dying in the 51st week is approximately 12%.

Sacher and Brues[7-11] first proposed that the hazard function is an exponential function of the mean intensity of physiological injury for laboratory rodents, i.e. inbred, homogeneous mammals, kept free of preventable disease and housed in a uniform environment,

$$h(x) = ke^{\phi(x)} \qquad (2)$$

where h(x) is the hazard function at age x, k is a proportionality constant related to the inability of the population to withstand injury from the environmental conditions and $\phi(x)$ is the mean intensity of injury of the population at age x. Sacher[5] proposed that $\phi(x)$ is a weighted summation of tissue injury, resulting in mortality, from the natural aging process as well as from environmental insults.

Taking Napierian logarithms of Eq. 2 yields the log-hazard function (Gompertz Transform or Gompertz function)

$$\ln[h(x)] = \ln k + \phi(x) \quad (3)$$

or

$$G_x = G_0 + \phi(x), \quad (4)$$

where, G_x is termed the Gompertz transform or Gompertzian[8] in honor of Benjamin Gompertz who demonstrated that age-specific mortality rate increases exponentially for human populations between ages 35 and 85.[12] In Eq. 4, G_x is the Napierian logarithm of the hazard function at time x. The parameter, G_0, is the extrapolated intercept at time 0, before the onset of aging. Typically, time 0 in toxicity studies is the age of weaning. Termed the vulnerability parameter, it is related to the initial vulnerability of the population in the environment (before the onset of aging) to causes of disease and mortality occurring during senescence[11,13].

Sacher and Trucco[14] were the first to relate the decline of physiological processes associated with aging with a change in the Gompertz function using Brownian motion for a free particle as a mathematical representation of the physiological state of an organism as it moves through a configuration space with a limiting hypersurface (lethal bound). They used a Gaussian distribution to represent the location of the frequencies of mean physiological states of a homogeneous population within the configuration space. They predicted that if the mean physiological state of a group of individuals declined linearly, then an exponential increase in the rate of mortality would follow. They emphasized, however, that their theory does not predict a linear decrease of physiological performance per se.[14]

Strehler and Mildvan[15,16] used a Maxwell-Boltzman distribution to characterize the magnitude (harmfulness) of environmental stresses-challenges. Their theory does predict a linear decline of physiological function that occurs with age and a corresponding exponential increase in human mortality between age 35 and 85.[16,17] Lestienne[18] integrated the concept of programmed longevity into Strehler and Mildvan's model. He characterized Gompertz distributions by the Gompertz slope and finite lifespan rather than the traditional Gompertz slope and Gompertz intercept.

Early on, there was disagreement concerning the mathematical form of ϕ (x). Sacher[8] postulated that a linear Gompertz function would only be seen in homogeneous, inbred, mammalian populations housed in well-controlled environments (e.g., rodents using in toxicity studies). He suggested that genetically heterogeneous populations, populations in heterogeneous environments, or populations exposed to environmental toxicants would likely exhibit a curvilinear Gompertz function. Homogeneous, inbred rodent populations housed in uniform, well-controlled environments and fed nutritionally adequate diets do exhibit the linear Gompertz function after weaning.[19-25] Use of the linear Gompertz function to model mortality of these animals helps to limit the number of parameters in potentially over-parameterized mathematical models of mortality. Other, highly parameterized, empirical models used to characterize the mortality of heterogeneous populations generate the linear Gompertz function as a special case.[26-35]

The linear Gompertz function is

$$G_x = G_0 + \alpha x, \quad (5)$$

where α is a first-order aging rate constant which characterizes the rate at which the hazard function associated with initial vulnerability (G_0) progresses with age (i.e., α characterizes senescent injury)[8]. Sacher[11] proposed that increments or decrements to injury resulting from exposure to exogenous agents are superimposable on senescent injury. Superimposition of responses is a principle widely used in pharmacokinetics[36]. Boxenbaum and colleagues[19,37] modified Gompertz (log-hazard) functions to characterize the time course of injury arising from acute and chronic exposure to environmental toxicants. Boxenbaum and Neafsey and colleagues[19-25] described discrete classes of perturbations to the linear Gompertz relationship resulting from irreversible injury, reversible injury, longevity hormesis and caloric restriction (occurring independently of one another). Boxenbaum *et al*.[19] also illustrated how a heterogeneous population would alter the Gompertz function in some cases.

Survival data published as cumulative mortality or percent survivorship plots were linearized with the use of the Gompertz transform to enable the identification and quantification of changes in mortality as they relate to dose and the temporal pattern of exposure. Unless otherwise noted (e.g., radon data), published plots of cumulative mortality or percent survivorship were digitized using the method described in Neafsey and Lowrie.[23] The graphs published in the original papers were photoenlarged. The enlargement process was tested for distortion by enlarging an accurately ruled piece of KandE graph paper. No measurable distortion was found. The graphs were digitized to an accuracy of 0.25mm to determine the number of deaths in each interval. This was

done three times. When totaled, these deaths agreed with the totals given in the original papers. The digitizing was rechecked by a second researcher. The error was estimated to be at most one death in an interval. The fact that the sum of the deaths in each interval equaled the published total deaths indicate that the original graphs were quite accurate.

The following Gompertz plots illustrate the shapes of temporal mortality fluctuations:
(1) acute, single-dose exposure, 2) chronic, fractionated toxicant exposure (no hormesis), 3) short-term toxicant exposure, 4) chronic, fractionated toxicant exposure (with hormesis), 5) combined toxicity and hormesis - lifespan exposure; 6) combined toxicity and hormesis - short-term exposure, 7) caloric restriction and 8) combined toxicity and caloric restriction.

RESULTS AND DISCUSSION

Acute, single-dose exposure: Figure 1 shows a Gompertz plot for male rats subjected to a single 0.5 Gy whole-body exposure of fission neutrons over a 22 hour period and sham-exposed controls (mortality data from Chmelevsky at al.[38]). Note that this short-term exposure to a single high dose of a toxicant instantaneously results in non-repaired injury that summates with aging injury. The modified linear Gompertz function for acute toxicity is:

$$G_x = G_0 + \alpha x + \varepsilon_D \quad (6)$$

where, ε_D is a term characterizing the additional irreversible injury at dose D. The addition of the permanent fixed injury term, ε_D causes a steady-state parallel upward displacement of the Gompertz function (Fig. 1).

In the 1950's Upton *et al.*[39,40] plotted parallel Gompertz functions of mice exposed to an experimental thermonuclear detonation (high-energy γ rays). Then Sacher[41] fit a quadratic equation (apparently a phenomenological choice) to the upward displacement of the Gompertz intercepts from this data set. He asserted that the parallel Gompertz functions indicated that the injury was not reparable and that the degree of upward displacement of the Gompertz functions indicated the degree of irreparable injury with dose.

Neafsey and Lowrie[23] fit Eq. 6 to the data of Chmelevsky *et al.*[38] and LaFuma *et al.*[42] from rats subjected to single whole body exposures to fission neutrons and gamma radiation, respectively.
They used a logarithmic-logistic function to relate ε_D to dose:

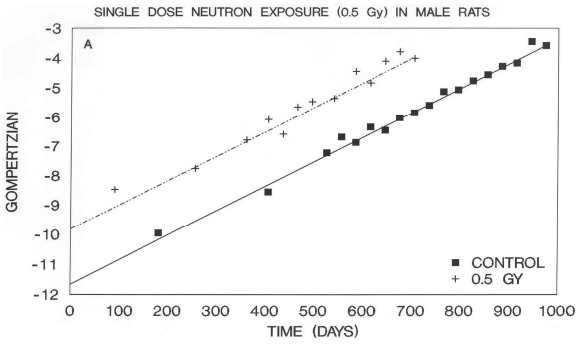

Fig. 1: Acute, Single-dose Exposure. Gompertz plot of Naperian logarithms of age-specific mortality rates for male Sprague-Dawley rats exposed to a single, whole body exposure of 0.5 Gy neutrons. Points are estimated Gompertzians calculated from the mortality data of Chmelevsky *et al.* [38] Time on the abscissa refers to that period following the initiation of the experiment at 3 months of age. Reproduced from Fig. 2 of Neafsey and Lowrie.[23] with permission of the copyright owner, Radiation Research Society

$$\varepsilon_D = \frac{\varepsilon_{max} D^{\tau}}{(\frac{1}{Q}) + D^{\tau}}. \quad (7)$$

By rearranging Eq. 7 they were able to use the model to estimate the dose of neutrons which produces a given displacement of the Gompertz function:

$$D = [\frac{1}{Q}\frac{\varepsilon_D}{(\varepsilon_{max}) - \varepsilon_D}]^{\frac{1}{\tau}} \quad (8)$$

and then calculate the relative biological effectiveness (RBE) of neutrons to γ radiation in terms of the degree of displacement:

$$RBE = \frac{D_\gamma}{D_N} \quad (9)$$

where, D_γ is the dose (Gy) of γ radiation for a given parallel upward displacement ε_D of the Gompertzian function and D_N is the dose (Gy) of fission neutrons for the same parallel upward displacement of the Gompertz function. Chmelevsky *et al.*[38] and LaFuma *et al.*[42] calculated RBE from their data using mean time to death with lung carcinoma. However, in their neutron studies, there were a greater number of competing causes of early death other than cancer (for example neurotoxicity,

hematopoietic toxicity and renal toxicity). For those toxicants with competing risks, age-specific mortality analysis is a useful adjunct because it integrates total causes of death as related to dose.

Chronic, fractionated toxicant exposure (no hormesis): Chronic (constant zero-order) exposure to a toxic agent (e.g. lifespan toxicity studies) that results in irreparable injury yields Gompertz functions with identical Gompertz intercepts but increased slopes indicating a constant age-independent enhancement (γ_D) of the mean intensity of injury:[8, 19-21]

$$G_x = G_0 + (\alpha + \gamma_D)x \quad (10)$$

where, γ_D is the cumulative toxicity with dose-rate D.

Neafsey *et al.*[21] studied lifespan exposure to 500-3500 ppm methylene chloride (inhaled 6 hours per day, 5 days per week) in female rats (raw mortality data from Burek *et al.*[43]). These mortality data generated a fan of Gompertz functions where γ_D was related to dose-rate by a logarithmic-logistic function (Fig. 2). The age-specific mortality rate analysis corroborates the morbidity data from the Burek *et al.*[43] study in which the exposed female rats showed significant increases in large and ulcerative benign mammary tumors.

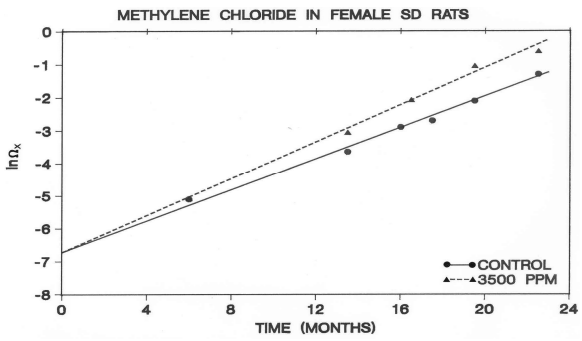

Fig. 2: Chronic, fractionated toxicant exposure (no hormesis). Gompertz plots of Napierian logarithms of age-specific mortality rates (estimated Gompertzians signified by ln Ω_x) versus time for control and methylene chloride-treated female SD rats. Methylene chloride vapor exposure (3500 ppm, 6 h/day, 5 days/week) was begun at 8 weeks of age and continued an additional 2 years. Time on the abscissa refers to that period following initiation of exposure. Original survival data were obtained from Burek *et al.* [43] Note that chronic exposure to toxic agents that do not induce hormesis increase the slope of the linear Gompertz function. Reproduced from Fig. 8 of Neafsey *et al.*[20] Permission of copyright owner (Marcel Dekker, Inc.)

Short-term toxicant exposure: When animals in toxicity studies are exposed for short periods of time and then allowed to live out their lifespans, Eq. 10 becomes

$$G_x = G_0 + \alpha x + (\gamma_D)b \quad (11)$$

where b is defined as the min (x, time of discontinuance). Figure 3A shows a Gompertz plot[25] for control and radon exposed male SPF Wistar rats. Exposure was to 1000-WL radon progeny; 15 mg m^{-3} uranium ore dust over 71.68 days for a total exposure of 10 240 WLM. Raw mortality data were available in tabular form from the third author of Neafsey, Lowrie and Cross.[25] The upward parallel displacement of the Gompertz curve after exposure ends ε_{ssD} (i.e. ε_D at steady state) was substituted for ε_D in Eq. 7. Figure 3B shows the ε_{ssD} from animals exposed at 1000-WL (90 h/week) for total doses from 320 WLM (2.24 days) to 10 240 WLM (71.68 days). Had the

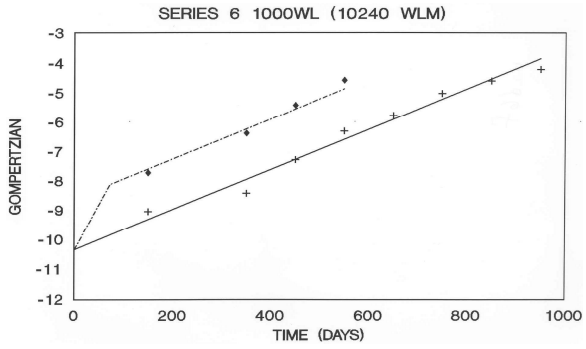

Fig. 3A: Short-term toxicant exposure. Gompertz plot of Naperian logarithms of age-specific mortality rates for control and radon exposed male SPF Wistar rats. Exposure was to 1000-WL radon progeny; 15 mg m^{-3} uranium ore dust over 71.68 days for a total exposure of 10 240 WLM[25]. Time on the abscissa refers to that period following the initiation of the experiment at 3 months of age. Reprinted from Fig. 2[25] of Mechanisms of Ageing and Development, Vol. 83, Neafsey, P.J., W.B. Lowrie and F.T. Cross FT, A mortality kinetics approach to characterizing the fractionated exposure-mortality response relationship of radon progeny, pp: 65-85, Copyright 1995 with permission from Elsevier

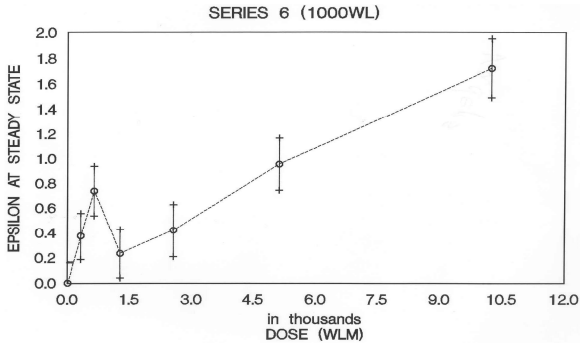

Fig. 3B: Displacement (ε_{ss}) of Gompertz curves with radon dose. Estimated degree of upward parallel displacement of Gompertz functions (ε_{ss}) at steady state for male SPF Wistar rats exposed to radon at 1000 WL (90 h/week) for total doses from 320 WLM (2.24 days) to 10 240 WLM (71.68 days). Points are observed ε_{ss} values and standard errors calculated form individual weighted least-squares analysis of Gompertzians from exposed and pooled control groups. The standard error of the Gompertz intercept is shown for pooled controls. Reprinted from fig. 5[25] of Mechanisms of Ageing and Development, Vol. 83, Neafsey, P.J., W.B. Lowrie and F.T. Cross FT, A mortality kinetics approach to characterizing the fractionated exposure-mortality response relationship of radon progeny, Pages 65-85, Copyright 1995 with permission from Elsevier

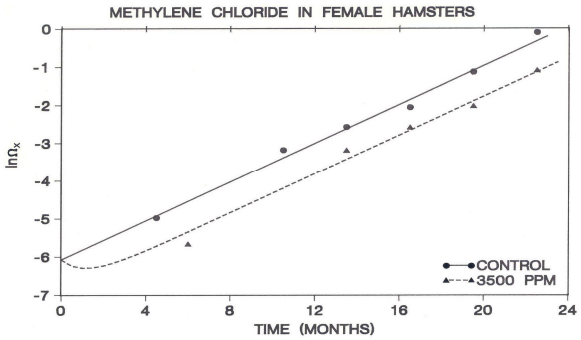

Fig. 4: Chronic, fractionated toxicant exposure (with hormesis). Gompertz plots of Napierian logarithms of age-specific mortality rates (estimated Gompertzians signified by ln Ω_x) versus time for control and methylene chloride treated female hamsters. Methylene chloride vapor exposure (3500 ppm, 6 h/day, 5 days/week) was begun at 8 weeks of age and continued an additional 2 years. Time on the abscissa refers to that period following initiation of exposure. Original survival data were obtained from Burek *et al*.[43] Note that the linear Gompertz function is displaced downward in a parallel fashion. Reproduced from Fig. 8 of Neafsey *et al*.[20] Permission of copyright owner (Marcel Dekker, Inc.)

lower dose 320 and 640 WLM exposures not been conducted, the striking linearity of the ε_{ssD} for the 1280, 2560, 5120 and 10 240 WLM groups might have led to the erroneous conclusion that the exposure-mortality response for radon exposure at 1000-WL is linear.

Chronic, fractionated toxicant exposure (with hormesis): Figure 4 shows a Gompertz plot for control and methylene chloride vapor-treated female hamsters.

Exposure was fractionated (3500 ppm, 6 h day^{-1}, 5 days/week) and begun at 2 months of age and continued for an additional 24 months (mortality data from Burek *et al*.[43]). At very low doses, hormetic agents displace the linear Gompertz function downward in a parallel fashion. Interestingly, the exposed hamsters had *higher* body weights than controls - a finding typical in studies of compounds that elicit longevity hormesis and absent in studies employing caloric restriction.[2-5,21] Histopathologic examination of the exposed hamsters demonstrated a methylene chloride dose-related decrease in geriatric changes and no toxic effects of methylene chloride.

A modified Gompertz function to accommodate longevity hormesis without toxicity resulting from chronic exposure to a hormetic agent is[19-21]

$$G_x = G_0 + \alpha x - \frac{\lambda_D}{K}(1 - e^{-Kx}), \quad (12)$$

where λD is the zero-order rate constant characterizing the reduction of injury at dose D. The constant K is a first-order rate constant characterizing the dissipation of the hormetic effect. The underlying assumptions are that: 1) hormesis produces decrements to aging injury and results in over-survival, 2) the hormetic effect occurs at a constant, age-independent rate and 3) hormesis is a reversible phenomenon - the benefits dissipate at a first-order rate so that when exposure to the hormetic agent ceases, the decrement in the Gompertzian dissipates exponentially after which the surviving organisms exhibit no actuarial benefit. Figure 6 (discussed below) illustrates this last point.

Combined toxicity and hormesis - lifespan exposure: Agents that elicit longevity hormesis (displacement of Gompertz function downward in a parallel fashion)

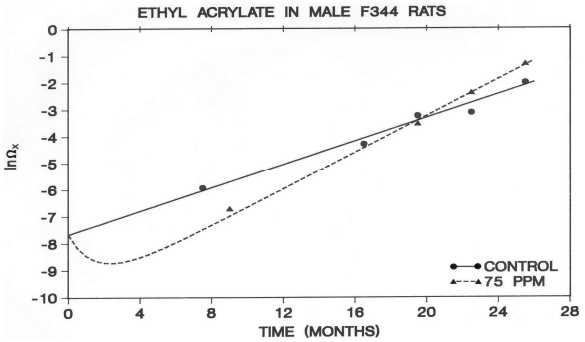

Fig. 5: Combined toxicity and hormesis–lifespan exposure. Gompertz plot of Napierian logarithms of age-specific mortality rates (estimated Gompertzians signified by ln Ω_x) versus time for control and ethyl acrylate vapor-exposed male F344 rats. Exposure (75 ppm, 6 h/day, 5 days/week) was begun at 7-9 weeks of age and continued for 27 months. Time on the abscissa refers to that period following initiation of exposure. Original survival data were obtained from Miller *et al*. [45] Note how the Gompertzians begin to increase over the controls after 20 months and produce the characteristic "silver spoon" perturbation to the linear Gompertz function. Reproduced from Fig. 6 of Neafsey *et al*. [20] with permission of copyright owner, Marcel Dekker, Inc

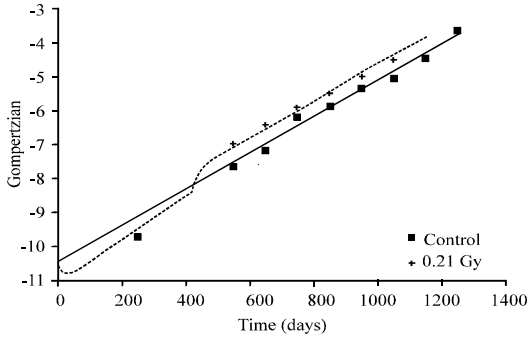

Fig. 6: Combined toxicity and hormesis–short-term exposure. Gompertz plot of Napierian logarithms of age-specific mortality rates for control sham-exposed and neutron exposed $B6CF_1$ male mice exposed to weekly doses of neutrons (420-day exposure period) of ~0.85 MeV mean energy, for a total dose of 0.21 Gy. Original mortality data are from Thomson and Grahn.[44] Note that there was no apparent increase in age-specific mortality rate during the 420 day period of fractionated exposures to fission neutrons. The Gompertzians of exposed animals exceeded those of controls only *after* termination of exposure. Reproduced from Fig. 3 of Neafsey and Lowrie [24] with permission of the copyright owner, Radiation Research Society

cause toxicity (increased Gompertz slope) at some dose. The effects of hormesis combined with the effects of chronic toxicity produce a characteristic silver spoon perturbation to the linear Gompertz function. Figure 5 shows a Gompertz plot for control and ethyl acrylate vapor-exposed male F344 rats (mortality data from Miller *et al*.[45]). Exposure was fractionated (75 ppm, 6 h/day, 5 days/week) and begun at 7-9 weeks of age and continued for and additional 27 months. The authors of the original study used cumulative mortality to compare exposed groups with controls and concluded that ethyl acrylate exposure did not adversely affect longevity of the mice. [45] Histopathologic examination of the mice revealed a lower incidence of medullary adrenal neoplasms (not statistically significant) and a significantly decreased incidence of benign tumors. The authors did not consider these tumor data to be of any toxicological significance. Note that age-specific mortality rate of the exposed animals does not increase over that of the control animals until 23 months and beyond. The NTP bioassay studies are only 24 months in length.

Using the principle of superimposition, the hybridized Gompertz function to accommodate the presence of combined longevity hormesis and cumulative toxicity over the life span is[20, 21]

$$G_x = G_0 + (\alpha + \gamma_D)x - \frac{\lambda_D}{K}(1 - e^{-Kx}) \quad (13)$$

Combined toxicity and hormesis – short-term exposure: Figure 6 shows a Gompertz plot[24] for control sham-exposed and neutron exposed $B6CF_1$ male mice exposed to weekly doses of neutrons (420-day exposure period) of ~0.85 MeV mean energy, for a total dose of 0.21 Gy (mortality data from Thomson and Grahn[44]). There was no apparent increase in age-specific mortality rate during the 420 day period of fractionated exposures to fission neutrons. The Gompertzians of exposed animals exceeded those of controls only after termination of exposure.

After the exposure ceased (420 days), a parallel upward displacement of the Gompertz functions (ε_{ssD})was

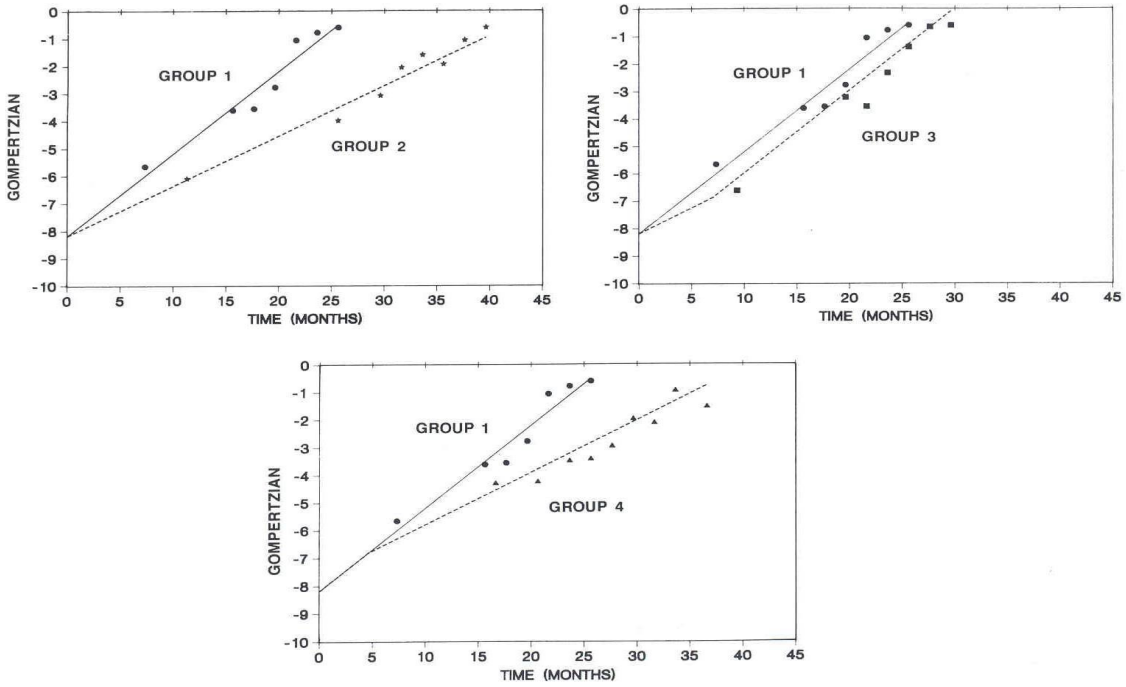

Fig. 7: **Caloric restriction. Top left panel:** Gompertz plots of Napierian logarithms of age-specific mortality rates versus time for control male F344 rats fed *ad libitum* (Group 1) and rats subjected to food restriction beginning at 6 weeks of age (Group 2). Food-restricted rats were fed 60% of the mean caloric intake of Group 1 rats until 18 months of age and maintained at that level until death. Top right panel: Gompertz plots of Napierian logarithms of age-specific mortality rates versus time for control male F344 rats fed *ad libitum* (Group 1) and rats subjected to food restriction from 6 weeks to 26 weeks of age (Group 3). Bottom panel: Gompertz plots of Napierian logarithms of age-specific mortality rates versus time for control male F344 rats fed *ad libitum* (Group 1) and rats subjected to food restriction beginning at 6 months of age (Group 4). Food-restricted rats were fed 60% of the mean caloric intake of Group 1 rats until 18 months of age and maintained at that level until death. Original mortality data were obtained from Yu et al. [46] Time on the abscissa refers to that period following the start of the experiment at 6 weeks of age. Note that the effect of caloric restriction is to decrease the slope of the linear Gompertz function. Reproduced from Fig. 1 of Neafsey et al. [22] with permission of the copyright owner, Marcel Dekker, Inc. .

apparent for all doses. Note that $\varepsilon_{ssD}/420=\gamma_D$ in Eq. (11). Neafsey and Lowrie[24] substituted ε_{ssD} for ε_D in Eq. 7 and 8 and calculated the RBE for neutrons compared to γ radiation for short-term exposure. A logarithmic-logistic function was used to relate ε_{ssD} with dose in order to calculate the relative biological effectiveness (RBE) estimates for fission neutrons.[24] Thomson and Grahn[44] used mean aftersurvival (MAS) as their index of cumulative effects of all injuries in the mice to estimate the RBE. Neafsey and Lowrie[24] demonstrated how the presence of hormesis during exposure confounded the calculation of the RBE estimates when mean after survival (MAS) was used as a response variable. Because MAS consolidates the entire mortality patter into a single variable, variations in mortality rates over time are masked. Use of the upward, displacement of the Gompertzians (ε_{ssD}), after exposure has ended offers an improved method for calculating RBE, a parameter intended to measure long-term toxicity.

Caloric restriction: Figure 7 shows Gompertz plots[22] for data from Yu *et al*.[46] Control male F344 rats fed ad libitum (Group 1) are compared to rats who were subjected to caloric restriction. They were given 60% of the mean intake of the controls until 18 months of age and maintained at that level until death (Group 2). Unlike animals that exhibit hormesis, these animals subjected to caloric restriction had a lower body weight than controls. This is a common finding[2-4,20,21,47]. Note that caloric restriction results in a *decreased slope* of the linear Gompertz function, suggesting a decrease in the senescent injury.

The Gompertz equation for caloric restriction is[22]

$$G_x = G_0 + (\alpha - \omega_D)x \quad (14)$$

where, ω_D is the parameter characterizing the decrement to the aging rate constant.

Yu *et al*.[46] examined median survival time and inspected the slopes of the survival curves. Because the survival curves of rats subjected to food restriction initiated in early life (6-26 weeks; Group 3) appeared to be parallel to that of the ad libitum fed group (Group 1) whereas the slope of the survival curve of the rats subjected to food restriction in adult life (Group 4) appeared to be different from that of the control group, Yu *et al*. concluded that food restriction during the development period increased longevity by a different mechanism than food restriction initiated in adult life. However, applying equation 14 to the data reveals that caloric restriction in rats initiated in early life and carried out either for a short period or the entire lifespan or initiated at maturity and carried out for the entire lifespan produces improved survival by decreasing the Gompertz aging rate parameter, i.e. Gompertz slope.

Combined toxicity and caloric restriction: Toxic substances can cause depressed food intake (inanition) in exposed laboratory rodents[4,20]. Thus, toxicant exposure can cause laboratory animals to experience caloric restriction. Since caloric restriction and chronic toxicity displace the linear Gompertz function in opposite directions (caloric restriction decreases the slope; chronic toxicity increases the slope), laboratory animals experiencing inanition from chronic exposure to a toxic substance may benefit from the health effects of the caloric restriction - thus clouding interpretation of the study. The use of pair-fed controls in chronic toxicity studies would permit separation of the effects of inanition (caloric restriction) from those due to hormesis and cumulative toxicity.

Figure 8 shows a Gompertz plot[20] of *ad libitum* and pair-fed control male Osborne Mendel rats exposed to 1800 ppm chloroform in water beginning at 7 weeks of age and continuing for an additional 26 months (mortality data from Jorgenson *et al*.[48]). Note that compared to the *ad libitum* fed controls, the pair-fed controls had a reduced Gompertz slope as was seen with caloric restriction in Fig. 7. Much of the improved longevity in the chloroform exposed animals is apparently due to caloric restriction, not hormesis. Jorgenson *et al*.[48] concluded that the improved median survival times in the chloroform treated rats and pair fed controls were solely due to leaner body composition since both of these groups had lower body weights

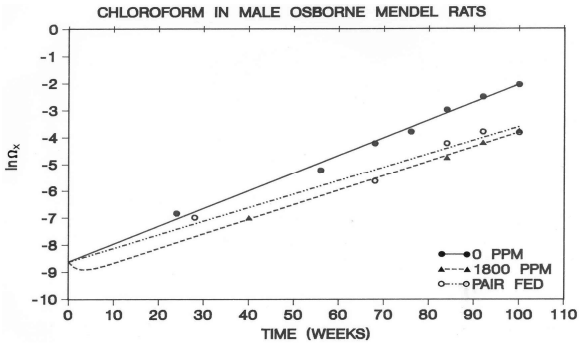

Fig. 8: Combined toxicity and caloric restriction. Gompertz plots of Napierian logarithms of age-specific mortality rates (estimated Gompertzians signified by ln Ω_x) versus time for 3 groups of Osborne Mendel rats: 1) controls fed *ad libitum* (0 ppm); 2) pair-fed controls; and 3) animals exposed to 1800 ppm chloroform for 104 weeks (26 months). Time on the abscissa refers to that period following initiation of exposure at 7 weeks of age. Original survival data were obtained from Jorgenson *et al*. [48] Reproduced from Fig. 9 of Neafsey *et al*.[20] with permission of the copyright owner, Marcel Dekker, Inc

compared to the untreated controls. In this study, inanition effects were of such magnitude that the toxic effects of chloroform on age-specific mortality rate were masked. That there were combined hormetic and toxic effects of chloroform was indicated by a lower than expected probability of death from renal tumors in the lower doses and an increased incidence of renal tumors in the highest dose, assuming a linear extrapolation of the probability of death.[48]

Proposed Mathematical Model to Analyze Longevity Data from Chronic Toxicity Studies.

The neoplastic and non-neoplastic endpoints seen in laboratory animals exposed to toxicants result from the interplay of effects from aging, depressed food intake, hormesis and toxicity. Future toxicity studies should be conducted over the life-span of the animals and include pair-fed controls and larger sample sizes. Age-specific mortality rate analyses would better enable toxicologists to identify the separate contributions of aging, depressed food intake, hormesis and toxicity to mortality in both chronic and short-term exposure studies[19-25].

The method proposed here[25] uses actuarial methods applied to the exposure-mortality response relationship. Often fractionated exposures are used in toxicity studies

wherein groups of animals are exposed at different dose rates for varying periods of time. The model has been applied to fractional exposure of male SPF Wistar rats exposed to radon progeny at 100 WL and 1000 WL for total exposures ranging from 20 to 10 240 WLM.[25] The method is reproduced here from Mechanisms of Ageing and Development, Vol. 83, Neafsey, P.J., W.B. Lowrie and F.T. Cross, A mortality kinetics approach to characterizing the fractionated exposure-mortality response relationship of radon progeny, Pages 72-74, Copyright 1995 with permission from Elsevier.

The exact time of death or sacrifice (if moribund) of each animal must be recorded and animals must be allowed to live out their life spans (typically 33 months for rats). The mortality data are used to calculate estimated Gompertzians. Each Gompertzian is given an estimated weight (W), which is the reciprocal of the variance. If there are too few deaths in an interval, the weight is too low and the value (if any) of the estimated Gompertzian function is subject to too much statistical variation. Therefore, in order to have estimated Gompertzians with a reasonably large weight during the early "silent" period when there are very few deaths, the early time periods (0-300 days) may be pooled. [23, 24] Gompertzians after the silent period are estimated at the midpoints of 100 day intervals (350, 450,…) where deaths represent at least 2% of the initial cohort numbers (but never fewer than 2 deaths).
The following equation is used:[24,41,49]

$$\ln \hat{\Omega}_x = \ln[-(\frac{1}{u_i})\ln \hat{P}_i], \quad (15)$$

where $\ln \hat{\Omega}_x$ is the Gompertzian estimate (i.e. G_x in Eq. 4) at the interval midpoint and is used to approximate the Gompertz function. The fraction of the population surviving the age interval with length $u_i = t_{i+1} - t_i$ is denoted as $\hat{P}_i$. For censored data, $\hat{P}_i = \hat{Q}_i$ where $\hat{Q}_i = d_i/(N_i - 1/2w_i)$, and where d_i and w_i are deaths and withdrawals during the interval, respectively. Actuarial statisticians Elandt-Johnson and Johnson[49] state that $\hat{Q}_i$ is approximately unbiased and the maximum likelihood estimate for the probability of death in the interval if the times at death are assumed to be distributed exponentially. The formula subtracts one-half of the interval for each withdrawal from the exposure, assuming that all withdrawals occur in the middle of the interval,[49] or that the withdrawals occur uniformly throughout the interval.

If the times of withdrawal during the interval are known, the heuristic argument for censoring can be extended. Then the corresponding proportion of the period for each withdrawal is subtracted. The Balducci assumption[50] used widely in actuarial work[51] is applied:

$$\hat{Q} = \frac{d_i}{N_i - \frac{1}{t_{i+1} - t_i}\sum_j w_{ij}(t_{i+1} - t_{ij})}. \quad (16)$$

where, the number of withdrawals in the interval (t_i, t_{i+1}) at assumed times $t_i \le t_{i1} < t_{i2} ... < t_{in} \le t_{i+1}$ are $w_{i1}, w_{i2},...,w_{in}$, respectively.

Unlike conventional (unweighted) least squares where it is tacitly assumed that variance estimates about each data point are equal, Gompertz analyses employ cohorts. Sacher[8,14] developed relationships to estimate the sampling variance where the statistical weights (W) used for $\ln \Omega_x$ values were the reciprocals of the estimates of the variance (V):[14,49]

$$W_i = \frac{1}{V_i} \cong \frac{[N_i \hat{P}_i (\ln \hat{P}_i)^2]}{(1 - \hat{P}_i)}. \quad (17)$$

This modified Gompertz model employs the treatment of concomitant variables (Johnson and Elandt-Johnson[49]). It is outlined and compared to the Cox model[52] in Neafsey and Lowrie[35].

The linear Gompertz function ($G_x = G_0 + \alpha x$) is fitted to the data for the control and exposed groups for the time period after the termination of exposure.

A nonlinear least-squares computer program PCNONLIN[53,54] or SAAM II[55] may be employed for curve fitting using initial estimates of parameters which may be obtained graphically. The Nelder-Mead[56] algorithm is used to search the parameter space. Other least-squares optimization software such as SAS, BMDP and SAAMII[57-59] have been used and yield parameter estimates which are essentially the same as those found by the Nelder-Mead algorithm. The Nelder-Mead algorithm has provisions for preventing the optimization method from getting stuck some distance from the global (local) minimum. The algorithm is very tolerant of poor initial estimates of the parameters at the expense of the relatively large number of iterations necessary for convergence. The Gauss-Newton method used in SAS (and also available in PCNONLIN and SAAM II) may diverge if the initial estimates are poor or the bounds on the parameters are too large.

Goodness-of-fit may be verified in three ways: 1) visual inspection of plots of weighted residuals versus x indicate randomness of scatter of data points about fitted curves,[60] 2) visual inspection of data points ($\ln \Omega_x$ - x pairs) about the regression lines indicate randomness-of-scatter,[61] and 3) the computed χ^2 values should be less than tabulated values ($\alpha = 0.05$).[6, 19]

Is the bottle half empty? The detection of hormetic effects elicited across species and substances[5] may lead some to "see the bottle as half-full" with respect to the implications of low-dose responses. However, toxicologists must ever be pessimistic, "see the bottle as half-empty" and err on the side of caution. The following changes in toxicity study design and data analyses are suggested in order to reveal the negative implications of hormesis for risk assessment:

Increase the number of dose levels and increase the number of animals at each dose: Calabrese and Blain [5] identified 45 NTP toxicity reports in which there was hormesis evidenced by a minimum of a 10% simulation of response in at least one dose when a depression was expected (e.g. growth) or a 3% depression in response when stimulation is expected (e.g. cancer). Simulations conducted by Michalski and Yashin[62] led them to assert that a minimum of 200 subjects are needed to detect a weak effect of hormesis in a homogeneous population (with a probability of more than 70%). If the NTP protocol employed 200 exposed animals (instead of the current 50), hormesis might be more evident. Rozman[63,64] argues that the number of animals could be greatly reduced if additional doses were added to define the temporal dose-responses. However, more research (both simulations and bioassays) must be conducted to delineate the optimal number of doses and animals at each dose.

Conduct chronic toxicity studies over the life-span: Typically chronic toxicity studies in rodents are conducted for 24 months. This is assumed equivalent to 70 man-years - but it essentially ignores the cumulative impact of exposure on the aging animal. For agents that produce hormesis with toxicity, the hormetic effect can mask toxic effects at early ages and (usually) at low doses. Could the lack of apparent toxicity at early ages (signified by equivocal pathological results) be due to the confounding effects of hormesis? If the 2-year protocol were changed to lifetime studies, would late-life toxic effects be evident from exposures that produced no significant toxicity at earlier ages?

Utilize pair-fed controls: Age-specific mortality data (and body weight data) suggest that caloric restriction and hormesis are two separate phenomena (despite the use of the terms interchangeably by several authors). The use of pair-fed controls in which a group of control animals are fed only that amount of food consumed by exposed animals needs to become standard practice in toxicity studies.

Since caloric restriction and chronic toxicity displace the linear Gompertz function in opposite directions (caloric restriction decreases the slope; chronic toxicity increases the slope), laboratory animals experiencing inanition from chronic exposure to a toxic substance may benefit from the health effects of the caloric restriction – thus clouding interpretation of the study[4,20,63]. The use of pair-fed controls in chronic toxicity studies would permit separation of the effects of inanition (caloric restriction) from those due to hormesis and cumulative toxicity.

Conduct combined exposure studies to more than one toxicant: If hormesis is a saturable mechanism in humans (due to the plethora of hormetic agents humans are exposed to in their environments) - or perhaps does not actually occur in humans and is a phenomenon only observable in laboratory settings - the implications are enormous. The hormetic mechanisms in humans may already be up-regulated to maximal levels.[65,66] Additional stresses from environmental agents that produce both toxic and hormetic effects might result in additional, cumulative toxic injury (especially at older ages) without additional hormetic dissipation of injury. If an agent is found to be hormetic at doses found in the environment, one cannot conclude these low doses are unlikely to contribute to the total hazard of exposed populations. Chronic toxicity studies should be conducted whereby several toxins that exhibit hormesis at low doses (e.g. dioxin[63] and any of the other agents studied by the NTP and identified by Calabrese and Blain[5] as hormetic agents) are administered separately and in various combinations over the lifespan of experimental rodents.

Employ age-specific mortality rate analyses to characterize temporal dose-response: Age-specific mortality rate analyses would better enable toxicologists to identify the separate contributions of aging, depressed food intake, longevity hormesis and toxicity to mortality in both short-term and chronic exposure studies. The method can assist the toxicologist in mathematically modeling mortality and time-dependent exposure to toxicants and can yield an index of net injury from all causes. With the employment of pair-fed controls and life-span studies, the separate and combined effects of hormesis and caloric restriction on mortality can be delineated. It offers a method to characterize the temporal pattern of net injury from acute, short term and fractionated or chronic environmental exposure and it can enable the identification of late-life effects during and following exposure. The method also offers an improved

technique over median after survival to estimate relative biological effectiveness of one agent versus another and characterize the combined effects of multiple agents.

REFERENCES

1. Calabrese, E.J., 2000. Introduction to the BELLE newsletter: Special issue on Caloric restriction and hormesis. Hum. Exp. Tox. 19: 319-384.
2. Rao, G., 1988. Rodent diets for carcinogenesis studies. J. Nutr., 118: 929-931.
3. Hayflick, L., 2001. Hormesis, aging and longevity determination. Hum. Exp. Tox., 20: 289-291.
4. NTP (National Toxicology Program), 1997. Effect of dietary restriction on toxicology and carcinogenesis studies in F344/N rats and B6C3F1 mice, NTP Technical Report (TR) 460, National Institute of Environmental Health Sciences, Research Triangle Park, NC.
5. Calabrese, E.J. and R. Blain, 2005. The occurrence of hormetic dose responses in the toxicological literature. The hormesis database: An overview. Tox. Appl. Pharm., 202: 289-301.
6. Lee, E.T., 1980. Statistical Methods for Survival Data Analysis. Lifetime Learning Publishers (Wadsworth), Belmont, CA.
7. Brues, A. and G. Sacher, 1952. Analysis of mammalian radiation injury and lethality. Symposium on Radiobiology: The Basic Aspects of Radiation Effects on Living Systems, Nickson, J.J., (Ed.), pp: 441-465. Wiley, New York.
8. Sacher, G.A., 1966. The Gompertz transformation in the study of the injury-mortality relationship: Application to late radiation effects and ageing. In Radiation and Ageing, Proceedings of a Colloquium Held in Semmering, Austria, June, 1966. Lindop, P.J. and G.A. Sacher (Eds.). pp: 411-441. Taylor and Francis, London.
9. Sacher, G.A., 1970. Models from radiation toxicity data. In Late Effects of Radiation. Fry, R.J.M., D. Grahn, M.L. Greim and J.H. Rust, (Eds.). pp: 233-244. Taylor and Francis, London.
10. Sacher, G.A., 1976. Dose, dose rate, radiation quality and host factors for radiation-induced life shortening. In Aging, Carcinogenesis and Radiation Biology Smith, K.S. (Ed.), pp: 493-517. Plenum Press, New York.
11. Sacher, G.A., 1977. Life table modification and life prolongation. In Handbook of the Biology of Aging. Finch, C.E. and L. Hayflick, (Eds.). pp: 582-638. Van Nostrand Reinhold, New York.
12. Gompertz, B., 1825. On the nature of the function expressive of the law of human mortality and on a new mode of determining life contingencies. Phil. Trans. R. Soc. Lond. A, 115: 513-585.
13. Sacher, G.A. and A.S. Hart, 1977. Longevity, aging and comparative cellular and molecular biology of the house mouse, Mus musculus and the white-footed mouse, Peromyscus leucopus. In Genetic Effects on Aging. Harrison, D.H., (Ed.). pp: 73-98. A.R. Liss, New York.
14. Sacher, G.A. and E. Trucco, 1962. The stochastic theory of mortality. Ann. NY Acad. Sci., 96: 985-1007.
15. Strehler, B.L. and A.S. Mildvan, 1960. General theory of mortality and aging. Science, 132: 14-21.
16. Mildvan, A.S. and B.L. Strehler, 1960. A critique of theories of mortality. In The Biology of Aging, Strehler, B.L., (Ed.). pp: 216-235. Publ. No. 6, Am. Inst. Biol. Sci., Washington, DC.
17. Strehler, B.L., 1977. Time cells and aging. 2nd. (Edn.). Academic Press, New York.
18. Lestienne, R., 1988. On the thermodynamic and biological interpretation of the Gompertzian mortality distribution. Mech. Ageing Dev., 42: 197-214.
19. Boxenbaum, H. C. McCullough and F. DiCarlo, 1985-1986. Mortality kinetics in chronic toxicity studies. Drug Metab. Rev., 16: 321-362.
20. Neafsey, P.J., H. Boxenbaum, D.A. Ciraulo and D.J. Fournier, 1988. A Gompertz age-specific mortality rate model of aging, hormesis and toxicity: Single dose studies. Drug Metab. Rev., 19 (3,4): 369-401.
21. Neafsey, P.J., H. Boxenbaum, D.A. Ciraulo and D.J. Fournier, 1989. A Gompertz age-specific mortality rate model of aging, hormesis and toxicity: Dose-response studies. Drug Metab. Rev., 20: 11-150.
22. Neafsey, P.J., H. Boxenbaum, D.A. Ciraulo and D.J. Fournier, 1989. Aging and the Gompertz function: Modification by food restriction in rats. Drug Metab. Rev., 21: 217-231.
23. Neafsey, P.J. and W.B. Lowrie, 1993. A Gompertz age-specific mortality rate model of toxicity from short term neutron whole-body exposure to fission neutrons in rats. Radiat. Res., 133: 234-244.
24. Neafsey, P.J. and W.B. Lowrie, 1994. A Mortality kinetics approach to characterizing the relative biological effectiveness of short-term exposure to fission neutrons. Rad. Res., 137: 208-225.
25. Neafsey, P.J., W.B. Lowrie, F.T. and F.T. Cross, 1995. A mortality kinetics approach to characterizing the fractionated exposure-mortality response relationship of radon progeny. Mech. Ageing and Devel. 83: 65-85.

26. Economos, A.C., 1980. Kinetics of metazoan mortality. J. Soc. Biol. Struct., 3: 317-329.
27. Economos, A.C., 1982. Rate of aging, rate of dying and the mechanism of mortality. Arch. Gerontol. Geriat., 1: 3-27.
28. Economos, A.C., 1985. Rate of aging, rate of dying and non-Gompertzian mortality - Encore. Gerontology, 31: 106-111.
29. Witten, M., 1983. A return to time, cells, systems and ageing: Rethinking the concepts of senescence in mammalian systems. Mech. Ageing Dev., 21: 69-81.
30. Witten, M., 1984. A return to time, cells, systems and ageing: Relational and reliability theoretic approaches to the study of senescence in living systems. Mech. Ageing Dev., 27: 323-340.
31. Witten, M., 1985. A return to time, cells, systems and ageing: III. Gompertzian models of biological aging and some possible roles for critical elements. Mech. Ageing Dev., 32: 141-177.
32. Witten, M., 1986. A return to time, cells, systems and ageing: IV. Further thoughts on Gompertzian survival dynamics. The neonatal years. Mech. Ageing Dev., 33: 177-190.
33. Witten, M., 1988. A return to time, cells, systems and ageing: V. Further thoughts on Gompertzian survival dynamics. The geriatric years. Mech. Ageing Dev., 46: 175-200.
34. Piantanelli, L., 1986. A mathematical model of survival kinetics. I. Theoretical basis. Arch. Gerontol. Geriat., 5: 107-118.
35. Piantenelli, L., F. Scerre, G. Cognini and G. Linari, 1986. A mathematical model of survival kinetics. II. Parameter estimation. Arch. Gerontol. Geriat. 5: 119-130.
36. Thron, C.D., 1974. Linearity and superposition in pharmacokinetics. Pharmacol. Rev. 26: 3-31.
37. Boxenbaum, H., 1992. Hypothesis on mammalian aging, toxicity and longevity hormesis: Explication by a generalized Gompertz function. In: Biological Effects of Low Level Exposures to Chemicals and Radiation. Calabrese, E.J., (Ed.). pp: 1-39. Lewis, Chelsea, MI.
38. Chmelevsky, D., A.M. Kellerer, J. LaFuma, M. Morin and R. Masse, 1984. Comparison of the induction of pulmonary neoplasms in Sprague-Dawley rats by fission neutrons and radon daughters. Radiat. Res., 98: 519-535.
39. Furth, J., A.C. Upton, K.W. Christenberry, W.H. Benedict and J. Moshman, 1956. Some late effects in mice of ionizing radiation from an experimental nuclear detonation. Radiology, 67: 250-257.
40. Upton, A.C., M.A. Kastenbaum and J.W. Conklin, 1960. Age-specific death rates of mice exposed to ionizing radiation and radiomimetic agents. In: Symposium on Cellular Basis and Aetiology of Late Somatic Effects of Ionizing radiations. Harris, R.J.C., (Ed.). pp: 285-294. Academic Press, New York.
41. Sacher, G.A., 1956. On the statistical nature of mortality, with especial reference to chronic radiation mortality. Radiology, 67: 250-258.
42. LaFuma, J., D. Chmelevsky, J. Chameaud, M. Morin, R. Masse and A.M. Kellerer, 1989. Lung carcinomas in Sprague-Dawley rats after exposure to low doses of radon daughters, fission neutrons, or γ rays. Radiat. Res., 118: 230-245.
43. Burek, J.D., K.D. Nitschke, T.J. Bell, D.L. Wackerle, R.C. Childs, J.E. Beyer, D.A. Dittenber, L.W. Rampy and M.J. McKenna, 1984. Methylene chloride: A two-year inhalation toxicity and oncogenicity study in rats and hamsters. Fundam. Appl. Toxicol. 4: 30-47.
44. Thomson, J.F. and D. Grahn, 1988. Life shortening in mice exposed to fission neutrons and γ rays, Radiat. Res. 115: 347-360.
45. Miller R.R., T.J. Young, R.J. Kociba, D.G. Keyes, K.M. Bodner, L.L. Calhoun and J.A. Ayres, 1985. Chronic toxicity and oncogenicity bioassay of inhaled ethyl acrylate in Fischer 344 rats and B6C3F1 mice. Drug Chem. Toxicol. 8: 1-42.
46. Yu B., E. Masoro and C. McMahan, 1985. Nutritional influences on aging of Fischer 344 rats: I. Physical, metabolic and longevity characteristics. J. Gerontol., 40: 657-670.
47. Van Voorhies, W.A., 2001. Hormesis and aging. Human and Exp. Tox., 20: 315-317.
48. Jorgenson, T.A., E.F. Meierhenry, C.J. Rushbrook, R.J. Bull and M. Robinson, 1985. Carcinogenicity of chloroform in drinking water to male Osborne-mendel rats and female B6C3F1 mice. Fundam. Appl. Toxicol. 5: 760-769.
49. Elandt-Johnson, R.C. and N. Johnson, 1980. Survival Models and Data Analysis. Wiley, New York.
50. London, D., 1986. Survival Models, Actex Co., Winsted, CT.
51. Bowers Jr, N.L., H.U. Gerber, J.C. Hickman, D.A. Jones and C.J. Nesbitt, 1986. Actuarial Mathematics, Society of Actuaries, Chicago.
52. Cox, D. and R.D. Oakes, 1984. Analysis of Survival Data. Chapman and Hall, London.
53. Statistical Consultants, Inc., 1986. PCNONLIN and NONLIN84: Software for the statistical analysis of nonlinear models. Am. Statistician, 40: 52.
54. Weiner, D.L., 1986. Nonlin84/pcnonlin: Software for the statistical analysis of nonlinear models. Meth. Find. Exp. Clin. Pharmacol., 8: 625-628.

55. SAAM II (Digital Ventures, U. Washington, Seattle).
56. Nelder, J.A. and R. Mead, 1965. A simplex method for function minimization. Computing J., 7: 308-313.
57. SAS User's Guide: Statistic, 1990. SAS Institute, Cary, NC.
58. BMDP Statistical Software Manual. Vols. 1 and 2, 1990. University of California Press, Berkeley, CA.
59. Heatherington, A.C., P. Vicini and H. Golde, 1998. A pharmacokinetic/pharmacodynamic comparison of SAAM II and PC/WinNolin modeling software. J. Pharm. Sci. 87 (10): 1255-1263.
60. Daniel, C. and F.S. Wood, 1980. Fitting Equations to Data: Computer Analysis of Multifactor Data, 2nd Edn. Wiley, New York.
61. Boxenbaum, H., S. Riegelman and R.M. Elashoff, 1974. Statistical estimations in pharmacokinetics. J. Pharmacokinet. Biopharmacol., 2: 123-148.
62. Michalski, A.I. and A.I. Yashin, 2002. Detection of hormesis in longevity: Simulation approach for heterogeneous population. Math. Biosci. 175: 57-66.
63. Rozman, K.K., M. Lebofsky and D. Pinson, 2005. Chronic toxicity and carcinogenicity of 1,2,3,4,6,7,8-heptachorodibenzo-p-dioxin displays a distinct dose/time toxicity threshold (c x t = k) and a life-prolonging subthreshold effect. Tox. Sci. Food Chem. Toxicol. 43 (5): 729-740.
64. Rozman, K.K., 2005. Hormesis and risk assessment. Human and Experimental Tox. 24 (5): 255-257.
65. Neafsey, P.J., 1990. Longevity hormesis: A review. Mech. Aging Devel. 51: 1-31.
66. Minois, N., 2001. Applying hormesis in aging research and therapy: a commentary. Human and Experimental Tox., 20: 309-310.