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Platform Sessions

Biphasic Dose Responses in the Biological Sciences

Edward J. Calabrese[1], University of Massachusetts

Two databases have been developed to assess the frequency of hormetic responses in the toxicological literature and their quantitative features. Using rigorous *a priori* entry and evaluative criteria hormetic responses occur in approximately 40% of experiments satisfying the entry criteria. The dose response characteristics of hormetic effects reveal that the maximum amplitude of the response is modest with maximum stimulatory responses usually 30-60% greater than controls, and rarely exceeding 100%. The range of the stimulation is more variable, with 75% less than 10-fold, with 5% exceeding 100-fold and about 1-2% exceeding 1000-fold. The mechanism of hormetic-like dose responses is well described in several dozen pharmacological receptor systems featuring a concentration gradient regulatory progress acting via opposite acting receptor subtype with different affinities for the agent. Biphasic dose responses are extremely common in essentially all biological systems and likely represent the most dominant biological dose response model.

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Implications of Non-Linearity for Ecotoxicological Risk Assessment

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Nonlinear concentration-responses are frequently observed in ecotoxicological bioassays. In the laboratory, these responses are similar to those observed in other bioassays and are usually manifest as increases in survival, size, biomass, or reproductive rates. In the context of the laboratory observations, relevance of these results to the field are difficult to interpret, although some of the observations may be examples of stress responses that are interpreted as positive responses by the observer. For example, internode length in the aquatic macrophyte *Myriophyllum* spp. is affected by stressors, usually increasing at smaller concentrations and decreasing at greater concentrations. The stimulation at low concentration is classically defined as hormesis, but it is also a natural stress response to lack of photosynthesis because of changes in light penetration into the water column, etc. Similar responses are observed in experimental field tests conducted in mesocosms or microcosms. Here the results may have nothing to do with the direct effect of the organisms demonstrating the hormetic response. Food-web interactions may result in release from predation or increased availability of food, both of which may be interpreted as non-linearity. The common belief that hormetic responses are “good” for the organisms must be interpreted in the context of the ecosystem where increases in numbers, either of predator or prey organisms, are not necessarily “good” for the community. Increases in populations of some organisms may be temporary and be followed by recover or regression to the mean. In this case, they can be interpreted as indicators of response in the community, however, their relevance in the long-term continuation of the community is reduced if recovery is rapid. Examples of these responses will be presented and their relevance in ecotoxicological risk assessment discussed.

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Evolutionary Foundations of Non-Linearity

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For eons, plants have been evolving the ability to synthesize complex chemicals aimed at deterring herbivores, while animals have evolved metabolic and physiological means to detoxify these chemicals. Examining the biological legacy of this battle is relevant to understanding physiological responses to modern synthetic chemicals. Taking an evolutionary perspective on hormesis means examining the circumstances under which natural selection should create and preserve the underlying nonlinear dose-response relationships; hence, it is tied to an examination of hormetic mechanisms. I examine three broad classes of proposed mechanisms: hormesis as stimulation of defenses or repair processes; hormesis as a consequence of homeostatic control; and hormesis as the net result of several simultaneous dose-related processes, some beneficial and others adverse. Understanding which changes are "beneficial" and which "adverse" can be problematic, and I examine how the perspective of Darwinian fitness may differ from that of the agronomist or the clinician. Evolving "fitness" has, however, conceptual similarities to the multiattribute decision theory of social scientists with its focus on optimal tradeoffs among different attributes to maximize overall utility. Expectations (in the statistical sense) about potential environmental challenges must be balanced with the costs of maintaining the means to combat them. The evolution of essentiality, the evolution of efficiency in resource utilization, and the role habitat selection, inter- and intra-specific competition, and environmental stress gradients are briefly discussed as they bear on the evolution of defenses against toxicity.

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Risk Assessment Implications of Non-Linear Dose Responses

William F. Greenlee[1], Frederick J. Miller[2], and Rory B. Conolly[3], CIIT Centers for Health Research.

Low dose extrapolations may be either linear or nonlinear, depending on the supporting database, but in either case are typically assumed to be monotonic for purposes of risk assessment. The recognition that some dose-response behaviors are not strictly monotonic but, rather, have "U", "inverted U", or even more complex shapes have important implications for toxicological research and testing as well as risk assessments. Among these are:

(1) Effects considered adverse may occur at doses lower than those associated with no effect.
(2) Small changes in endpoints normally considered adverse may reflect adaptive responses that are not adverse per se.
(3) Traditional experimental designs likely do not include enough dose levels to detect complex low dose behaviors that depart from expected monotonic behaviors.
(4) Research in support of biologically based dose-response models should carefully evaluate homeostatic and adaptive processes that may lead to nonmonotonic dose response behaviors at low doses.

While the existence of nonmonotonic low dose behaviors is not in doubt, the detection, appropriate interpretation, and ability to predict occurrence of these behaviors depends on an adequate understanding of the underlying biology. Dose-response curves for adverse endpoints such as cancer or developmental anomalies arise from the interplay of multiple factors, such as changes in levels of gene expression, in amounts and activities of signaling molecules, and in the behaviors or regulatory networks, all of which exist at more basic levels of biological organization than the manifestation of the toxic effect. Only when biomedical science understands the normal biological processes that are the substrates for toxicological mechanisms and toxicological science understands these mechanisms in the context of whole body physiology, will a scientifically mature understanding of complex dose-response behaviors be possible. This increased scientific sophistication will support identification of sensitive in vitro and in vivo biomarkers in laboratory animals and people, appropriate interpretation of the health significance of these biomarkers, guide the development of statistical criteria for experimental work, and enable development of predictive computational models of the dose-response relationship.

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Complex Shapes of Dose-Response Curves as the Summation of Underlying Low-Dose-Linear and Saturable Processes

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The shapes of dose-response curves for adverse effects such as cancer and for complex biochemical events such as signal transduction and gene expression are determined by the interactions of multiple underlying processes. With cancer, for example, a linear dose-response for DNA damage can combine with inducible but saturable DNA repair to generate a variety of nonmonotonic and monotonic dose-response shapes for tumor incidence. With the steroid hormone nuclear receptor system, differences in the relative affinities of ligand homo- and heterodimers for a limited number of promoter sites on the DNA can lead to a variety of dose-responses for gene expression. Computer simulation models of these systems will be used in this presentation to illustrate how complex dose-response behaviors arise from underlying linear and linear-saturable processes. Laboratory data consistent with the models will be presented, and statistical issues in the design of experiments to identify the behaviors will be considered.

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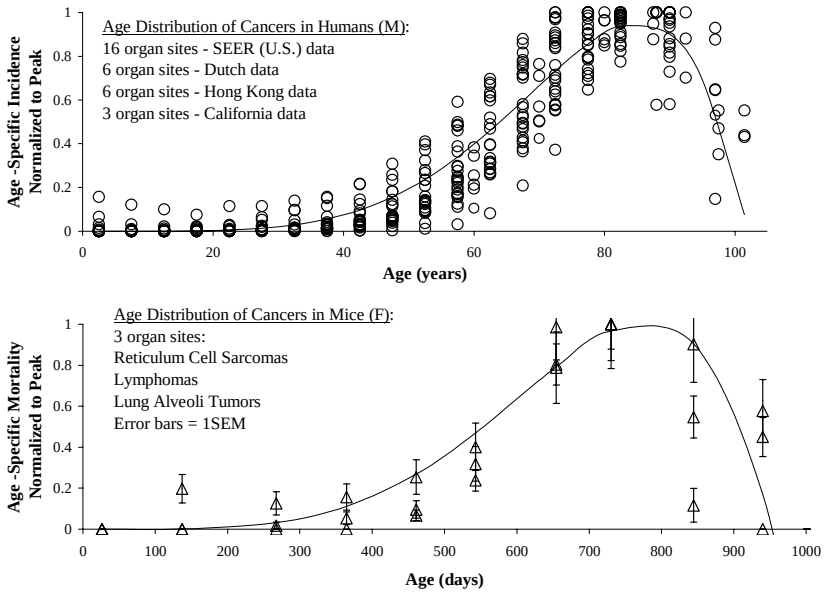
From Mice to Men, Cancers Are Not Certain at Old Age

Francesco Pompei[1], Harvard University; Richard Wilson[2], Harvard University

We have studied age-specific incidence data in humans from SEER (U.S.), Holland, Hong Kong, and California and found that organ site incidences do not continue to increase with age, but tend to peak at about age 80, and reduce thereafter toward zero at age about 100. We have also studied cancer incidence (age-specific mortality for lethal cancers) in mice as a function of age in the ED01 undosed controls study cohort ("megamouse" 2-AAF study), where they have been allowed to live very close to their full natural lifetime, and found that similar turnover in incidence occurs at about age 800 days, or 80% of lifetime. A Beta function model [$I(t) = (\alpha t)^{k-1}(1-\beta t)$; α, β, k are constants] fits both the mouse data and the human data well. The model suggests that increasing replicative senescence with age might be the cause of the turnover at old age. Limited data from National Toxicology Program mice studies suggest that the cancer age distribution, including the turnover, may be time-shifted by dietary restriction. Results of a large rat cohort study ("two tons of rats" nitrosomine study) were inconclusive since the rats did not live long enough for cancer incidence to either turn over, or to reach certainty.



Incidences are normalized to the peak value for each organ site. Organ site incidence data varied over two orders of magnitude for humans, and a factor of three for mice.

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Implications of Hormesis in Developmental Toxicology Risk Assessment

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In order to estimate the risk of an adverse effect to the offspring of individuals, as a result of exposure to a toxic substance during pregnancy, a common approach is to fit a dose-response model to animal bioassay data and use extrapolation to obtain upper confidence limits on risk for a fixed low exposure level. Equivalently, a lower confidence bound on exposure may be found for a negligible low level of risk. Often a monotonic dose-response function is used to express the relationship between the exposure level and probability of an adverse effect. Recently, however, there has been some evidence to suggest that it is not uncommon to experience a hormetic effect in developmental toxicology experiments. Here, we consider the implications that a non-monotonic dose-response model will impose on risk assessment procedures in developmental toxicology. We examine the current risk assessment paradigm and explore potential roles that hormetic dose-response models can legitimately play in different stages of that paradigm.

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Using Dose and Time to Predict Acute and Chronic Toxicity

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Groups of 30 to 60 adult female rats were administered singe doses of 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin (HpCDD) ranging from 1.0 to 10.0 mg/kg in corn oil (4 ml/kg) by gavage. Other groups of 30 females received a loading dose followed by biweekly maintenance doses to provide kinetic steady state. Body weight and feed intake were monitored at least 5 times per week. Animals were observed daily for signs of wasting/hemorrhage, anemia and lung cancer (visible as an abscess upon death). The different effects were allowed to run their time course unless rats were in severe distress in which case they were euthanized. Upon death, heart, lungs, kidney, upper G.I. tract, sternum and femur, liver and any macroscopically observable tumors were harvested, embedded, sectioned and stained with H&E. Microscopic examination confirmed in every instance macroscopic observations regarding anemia in that bone marrow cellularity was always less than 20% in exceptionaly pale rats. Additional macroscopically not identifiable squamous cell carcinomas of the lungs were also detected by light microscopy. Acute toxicity (wasting/hemorrhage) after single oral dose could be predicted according to dose x time = k or dose x time = k · W (W=effect) with less than 5% variability. Chronic toxicity (anemia plus lung cancer plus liver cancer plus various nephropathies) also obeyed the same laws of toxicology with even less variability. The repeated dosing regimen also provided exact dose x time predictions with the notable difference that the dose- and time-responses became steeper and all other chronic effects were compressed into anemia and lung cancer.

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Experiences in Non-Linear Dose-Response Relationships in Chemical Evaluations

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The Agency for Toxic Substances and Disease Registry (ATSDR) has legislative mandates to (1) identify chemicals of concern most commonly found at hazardous waste sites; (2) evaluate the public health implications associated with exposure to those chemicals in the environment; and (3) identify levels of significant human exposure, defined as Minimal Risk Levels (MRLs), for those chemicals. Although MRLs are usually derived from traditional dose-response data for the most sensitive toxicological endpoint, either in humans or the most sensitive animal species, some chemicals have been found to demonstrate non-linear responses at low-level exposures. Most notable of these type responses are chemicals which are known to be essential nutrients or otherwise demonstrate beneficial health effects at low-level exposures. In addition, preliminary evaluation of data from studies of infants exposed to low-levels of organic mercury in the form of ethyl mercury in vaccines suggests a dose-related increase in odds ratios for four separate neurological endpoints. These data also suggest an apparent threshold, with non-linear responses (decreased odds ratios) at sub-threshold exposure levels. The implications of these non-linear responses are presented and discussed relative to evaluation of the data for the purpose of developing chemical-, route-, and duration-specific MRLs.

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Risk Modeling Implications of Mechanistic Differences between Low and High Dose Effects of Arsenic

Christopher S. Wells[1], Gradient Corporation, Tracey M. Slayton[2], Gradient Corporation, Barbara D. Beck,[3] Gradient Corporation, Thomas A. Lewandowski[4], Gradient Corporation

Several lines of evidence suggest that the dose-response relationship for arsenic carcinogenesis, particularly at low doses, is likely non-linear. A number of in vitro arsenic toxicity studies show that treatment of cultured cells with low doses of arsenic (0.1 to about 10 μM) stimulates cell proliferation or increased cellular viability, whereas higher doses of arsenic increase cytotoxicity. Very low doses of arsenic have been shown to induce cellular protective mechanisms. Treatment of human keratinocytes with micromolar concentrations of arsenic upregulates genes relating to DNA excision repair, oxidative stress, and cellular redox control. In several studies, cultured cells treated with low doses of arsenic (0.5 to 10 μM) were more resistant to subsequent treatment with higher levels of arsenic or other toxic agents. The non-linearity of these cellular effects related to carcinogenesis may qualitatively scale to the level of whole organisms. Treatment of rats with 75 ppm arsenic in drinking water, both alone and in chemical mixtures, antagonizes the development of tumor precursor lesions. Epidemiological data on bladder and lung cancers in human populations exposed to a wide range of doses of arsenic (10 ppb to about 960 ppb) are best fit with non-linear biologically based models, according to Bayesian model selection approaches. Together, these findings provide support for the common scientific consensus on a non-linear dose-response relationship for arsenic carcinogenesis, and should be considered in the development of risk models. It is plausible that the toxicological consequences of low doses of ingested arsenic may be misstated by using linear models that disregard existing mechanistic data. Non-linear models may more accurately predict effects at lower, more environmentally relevant, arsenic doses.

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Hormesis, Low Dose Carcinogenicity and Low Dose Anti-Carcinogenicity Occur in the Same Animal and with the Same Chemical

Richard Wilson[1], Harvard University

Many authors tend to consider that if a chemical substance or other carcinogenic agent shows anticarcinogenicity at low doses or hormetic effects, then that is the only effect of that substance. However, I argue that this is an overly simplistic view. There are, for example, data (Kociba et al.) where 2,3,7,8 dioxin was fed to rats and the low dose effect was a reduction in all tumors. At higher doses, liver tumors increased although other tumors continued to decline. The carcinogenic/anticarcinogenic effect is unequivocal although the low dose reduction is often considered to be a spurious artifact. In the ED01 study, 2-AAF induced liver tumors with an approximate linear behavior but bladder tumors displayed a threshold. Not so well recognized is that the data, if they are believed, show a hormetic effect for reticular cell sarcoma. In earlier papers we have shown that the CBDS data base of the National Toxicology Program shows that a number of chemicals show significant anticarcinogenic responses (20%-30%) as carcinogenic ones (40%-50%), and these cannot be attributed to ordinary statistical sampling behavior. Many chemicals show both. The additive result of common tumors being reduced and rare tumors being increased is a net hormetic behavior. Implications for understanding of human cancer risks will be discussed.

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Data for Trichloroethylene-induced Kidney Tumors in Rodents Suggest an Epigenetic Mechanism of Action

Amy L. Lavin[1], Catherine F. Jacobson[2], and John M. DeSesso[3], Mitretek Systems, Inc.

Controversy exists regarding the potential of trichloroethylene (TCE) to cause kidney cancer in humans. This controversy is exemplified by the ongoing evaluation of the issue by the U.S. Environmental Protection Agency. An assessment of robust epidemiology data fails to support an association of TCE exposure at environmentally relevant levels with kidney cancer incidence. A lone exception is a study in cardboard factory workers purportedly exposed to extremely high concentrations. Chronic, high-dose TCE exposures in rats, but not mice or hamsters, have been shown to induce kidney tumors. The slightly elevated renal tumor incidences in the animal studies have always been seen in association with high incidences of chronic nephrosis and renal cytomegaly. The constant presence of nephrotoxicity in animals dosed chronically with high levels of TCE, in combination with mechanistic data showing TCE to be nongenotoxic, indicates an epigenetic mode of action. Furthermore, these data suggest that extrapolation of effects observed at high exposure levels to low-dose exposure scenarios is inappropriate. Based on this review of the data, it is concluded that a non-linear approach must be used to derive credible safe exposure levels for humans.

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Quantitation of the Dose Response for Formation of DNA Adducts in Rat Liver by 2-Acetylaminofluorene

Gary M. Williams[1], Michael J. Iatropolous[2], Jian-Dong Duan[3] and Alan M Jeffrey[4], New York Medical College

This study examined the dose response for AAF-DNA adduct formation in rat liver at two exposures below 28.2 mg/kg which did not produce preneoplastic lesions in a previous study and at 94.1 mg/kg and above, which produced promotable neoplasia in the previous study.

AAF exposure produced two major adducts: the deacetylated C8-dG-AF and N^2-dG-AAF, identified based on sensitivity to nuclease P_1 digestion and chromatographic properties and several minor adducts chromatographically similar to N^2-dG-AAF. After 12 weeks of exposure to AAF, the lowest dose group (0.92 mg/kg) showed no detectable C8-dG-AF adducts (<1 in 10^{10} normal nucleotides), but there was radioactivity associated with the area of N^2-dG-AAF adducts which appeared characteristic of AAF-DNA adducts but was not quantitatively different from the controls since a similar radioactivity/spot was also present in the control animals. The total number of adducts increased with exposure and after cessation of exposure, decreased. The ratio of N^2-dG-AAF to C8-dG-AF adducts increased over time and was inversely proportional to dose, ranging from 0.4 after a single exposure to AAF to >30 at 16 weeks.

Thus, under the conditions of this experiment, the low cumulative exposure of 0.92 mg/kg over 12 weeks was a no-observed-effect level for C-8-dG-AF DNA adduct formation (<1 in 10^{10}). For the N^2-dG-AAF adduct formation, we are still trying to resolve the extent to which olive oil contributes to this minor spot.

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Impact of Cellular Defense Mechanisms and Bystander Effects on a Multi-stage Carcinogenesis Model

Helmut Schöllnberger[1], University of Salzburg, Margaret G. Ménache[2], University of New Mexico, Rob Stewart[3], Pacific Northwest National Laboratory, Werner Hofmann[4], University of Salzburg.

The shape of dose-response curves for cancer incidence may be fundamentally altered by damage-induced adaptations in cellular defence mechanisms and by signal-mediated changes in cells that are not directly damaged by radiation (i.e., bystander effects). We have published a 3-stage clonal expansion model that is conceptually similar to the multi-stage cancer models proposed by Moolgavkar and others, except our model includes refinements to account for the hypothesized up regulation of DNA repair and radical scavenging in response to radiation damage. This cancer model successfully explains the hormesis effects suggested by some epidemiologic studies of lung cancer mortality [Schöllnberger *et al.*, Human and Ecological Risk Assessment, 7(4), 867-890, 2001].

A perennial challenge in the ongoing effort to develop mechanism-based carcinogenesis models is the lack of suitable datasets to rigorously test hypothesized mechanisms of action. In this presentation, strategies to incorporate radiologically induced cellular defence mechanisms and bystander effects into multi-stage cancer models are discussed. Then, the results of some modelling studies that examine the potential impact of these phenomena on cancer incidence rates as a function of dose and dose rate are presented. These studies are designed to test the feasibility of using data from epidemiologic studies alone to differentiate among hypothesized mechanisms of action.

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Human Cells Respond to Changes in Background Radiation by Inducing Specific Heat Shock Protein Members

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Accumulating evidence suggests that exposure of mammalian cells to low-doses of radiation induce cellular responses that modulate the level of damage caused by subsequent radiation exposure. Induction of these responses to low-doses of radiation may have serious implications for human health. However, the cellular responses evoked in human cells by varied radiation exposures within the range of natural background radiation are not fully known. Recent studies highlighted the importance of heat shock proteins (Hsps) as an effector of the apoptotic response in cells exposed to low-doses of radiation. We investigated the expression of Hsps in responses of normal human cells exposed to radiation environments at (~2 mG/y), or below (0.4 mG/y) that of natural background. Cultures of normal human fibroblasts (FBs) and bronchial epithelial cells (BECs) were maintained in normal and reduced background radiation environments for a total of 10 passages. Immunoblot analyses of total protein from FBs and BECs revealed cell-specific alterations in Hsp expression in cells grown in reduced background radiation environment. While FBs responded to changes in background environment by inducing Hsp90 and Hsp70, these proteins were not altered in BECs. Instead, high basal levels of Hsp27 were observed in BECs grown in reduced radiation background environment. The reduction of apoptosis has been suggested as one of the mechanisms of Hsp-mediated radio-adaptive responses. We next investigated whether alterations in Hsp levels modulate apoptotic responses of FBs and BECs to subsequent ionizing radiation (IR) exposure. Apoptosis was assessed by DNA fragmentation analysis. Compared to normal radiation environment, IR (10cGy) -induced apoptosis induction was significantly inhibited in Hsp overexpressing FBs and BECs grown in reduced background radiation environment. These studies suggest that normal human cells respond to changes in background by inducing specific Hsp family members that modulate the level of damage caused by subsequent radiation exposure.

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Low-Dose Protective Mechanisms: Implications for Risk Assessment

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Our research has focused on modeling stochastic effects induced in cells by low doses of genotoxicant agents. Using genomic instability state (GIST) models and Bayesian analyses, we can predict induced mutation, cell killing, and neoplastic transformation frequencies after low doses. Our most advanced GIST model is NEOTRANS$_2$. It includes both hypersensitive and resistant cells with mainly hypersensitive cells affected by low doses. NEOTRANS$_2$ also includes pathways for induction of genomic damage, repair (error-free), misrepair, apoptosis, and necrotic death. This model is being used to investigate the validity of the linear no-threshold (LNT) hypothesis as it relates to the induction of excess problematic nonlethal mutations and excess neoplastic transformations. Based on our modeling research, the LNT hypothesis cannot be valid if any of the following protective effects occurs at low doses: (1) no repair errors below a threshold dose rate; (2) induced additional repair mechanisms above a threshold damage level; or (3) an induced bystander effect for apoptosis that protects from problematic nonlethal spontaneous mutations and spontaneous neoplastic transformations. Using NEOTRANS$_2$, we show that low-dose induced protection against spontaneous mutations and neoplastic transformations via the postulated bystander effect for apoptosis leads to a stochastic threshold (StoThresh) dose. For the indicated stochastic effects, relative risk below the StoThresh is < 1, in contrast to values > 1 (as would be expected based on the LNT hypothesis). We also show that the postulated protection (via a bystander apoptosis effect) from spontaneous stochastic effects after low doses is consistent with observed hprt mutation induction in T-lymphocytes of mice indirectly exposed to ethylene oxide (via inhaled ethylene) and with in vitro data for radiation-induced neoplastic transformation. Our results indicate that reliance on the LNT hypothesis for low-dose risk assessment of stochastic effects can lead to greatly overestimating the actual risk. (Research supported by the U.S. Department of Energy, Offices of Science and Environmental Management).

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Non-Linear Dose-Response Curves in the Immune System Following Whole-Body X-Irradiation

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Since the first observation by the author's group in 1979 of immunologic stimulation among inhabitants in an area of high natural radioactivity in China, systematic experimental studies on immunity have been made in mice and in cell lines with multi-parameters after X-irradiation with 10 dose points ranging from 0.025~6.0 Gy. A total of 51 immunologic parameters were assessed in more than 1200 mice and other biological systems. With immunoehancing parameters an inverted J-shaped curve was observed with most significant up-regulation in immune functions at doses of 0.05 and 0.075 Gy, with an average increase in amplitude by 37.4% and 53.4%, respectively, and suppression at 4 and 6 Gy with an average decrease in amplitude by 56.1% and 63.9%, respectively. With immunodepressing parameters a J-shaped curve was usually found with most significant down-regulation of specific parameters at a dose of 075 Gy which caused a depression by 32.3% in average and most significant up-regulation at 2 Gy which caused a stimulation by 239.6% in average. The T lymphocytes were most significantly activated by low dose radiation with a tendency of preferential differentiation toward the Th1 subtype. Functional tests showed enhancement of anti-cancer immunity of the animals after low dose radiation. The results may well explain the observation of the significant decrease in growth rate and metastasis of implanted malignant tumor cells in mice exposed to whole-body X-irradiation with low doses. These experimental studies may lead to the conclusion that not all radiation is harmful and the influence of immunoenhancement has to be considered in the assessment of low level radiation effects on health.

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The Hormetic Health Effects of Radiation Observed in the Incident of Co-60 Contaminated Apartments in Taiwan

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There was an incredible radiological incident happened in Taiwan. About 1700 apartments were contaminated with Cobalt-60, and about 10,000 residents in the contaminated apartments had received quite amount of gamma radiation dose averaged in about 0.34 Sv, highest up 7 Sv until 2000. Based on the RERF report and ICRP publication, such amount of radiation could induce the residents to have many times of excess spontaneous leukemia and some excess solid cancer deaths, actually no excess leukemia and solid cancer deaths were observed, on the contrary, the overall spontaneous cancer deaths of the residents were sharply reduced to only 3.6 % of the general population. So that the radiation received continuously or chronically (nomenclature hereafter as chronic radiation) in the Co-60 contaminated apartments is always hormetic and could effectively immune from cancers. It is different from the health effects of radiation received instantaneously or acutely (nomenclature hereafter as acute radiation) in the nuclear explosion or accident that could cause higher cancer mortality, cause injure syndrome, and even cause death in higher doses. As chronic radiation is very much similar to the radiation received in the peaceful use of nuclear energy and medical use of radiation, chronic radiation should never be afraid by public but should be earnestly and medically employed as immunity from cancers, and it might also immune from hereditary diseases. The conventionally policies,

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standards and measures for radiation protection should be managed separately for benefiting not only the peaceful use of nuclear energy and medical use of radiation, but also for effectively used as immunity from cancers and hereditary diseases. The hormetic health effects of chronic radiation might also occur in other substances, such as toxic chemicals and microorganisms, it might conclude that any toxic substances received in low dose rate is always beneficial to humanity even in quite amount dose.

Dose-Response Relationship: Chromosome Aberrations in Residents at the High Background Radiation Areas in Ramsar, Iran

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There is not yet a solid dose-response relationship for chromosome aberrations induced by chronic exposure to elevated environmental levels of radiation in human. Among potential study populations, mostly occupationally exposed individuals, residents in areas with elevated levels of natural radiation may provide the most reliable information because more accurate dosimetry is possible and, unlike accidental exposures, non-radiation factors can be omitted. A linear relationship between the cumulative dose and the frequency of chromosome aberrations have been reported in residents at the high background radiation areas in China. People in some areas of Ramsar, a city in northern Iran, receive an annual radiation absorbed dose from background radiation that is substantially higher than the 20 mSv yr^{-1} that is permitted for radiation workers, and radon levels in some regions of Ramsar are up to 3700 Bq/m^3 (over 100 pCi/L). For example, the residents of one dwelling in Ramsar, receive doses estimated to be at least 160 mSv yr^{-1}, that is eight times higher than the dose limit for radiation workers. The people living in these high radiation areas are of considerable interest because they and their ancestors have been exposed to abnormally high radiation levels over many generations. Using chromosomal aberrations as the main endpoint, we carried out an experiment to assess the dose-effect relationship in the residents of high background radiation areas of Ramsar. A cytogenetical study was performed on 21 healthy inhabitants of the high background radiation areas and 14 residents of a nearby control area. Our preliminary results show no positive correlation between the frequency of chromosome aberrations and the cumulative dose of the inhabitants.

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Residential Radon in U.S. Counties vs. Lung Cancer in Women who Predominantly Never Smoked

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Previous observations that mean-residential-radon (Rn) levels for U.S. counties exhibits a negative ecologic association with age-adjusted county rates of lung-cancer mortality (LCM) may be erroneous because of either (I) confounding unaddressable by any county-level ("ecological") study design, or (II) county-level factors such as Rn/smoking or age/radon correlations or exposure misclassifications from the use of disparate data sources. To address issues II, age-specific LCM rates for white women in 2,821 U.S. counties who died in 1950–54 at age 40+ (~11% of whom ever smoked), or at age 60+ (~5% of whom ever smoked), were compared to county Rn levels newly estimated from U.S. Rn, climatic and geological-survey data. After adjusting for age and subsets of 21 county-level socioeconomic, climatic and other factors, significant negative LCM vs. Rn trends were found for both age groups, particularly among counties with ≤100 Bq m^{-3} Rn ($p \leq 0.00087$). But relative risk (RR_{adj}) for LCM was significantly elevated ($1 < [95\%$ conf. limits on $RR_{adj}] \leq 1.46$) in 20% of 210 comparisons made of LCM in counties with >150 vs. 65–100 Bq m^{-3} Rn—comprising the first reported ecological evidence of increased LCM risk due to radon based on U.S. county data. Analyses showing significantly increased risk all involved adjustment for for climatic and other factors likely to have influenced exposure to indoor air contaminants such as Rn and (secondary) cigarette smoke. Results from this study are most consistent with a nonlinear relation between 1950–54 LCM and U.S. residential radon among white women who predominantly never smoked.

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Treatment of Confounding Factors in Ecological Study Test of Linear-No Threshold Theory

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Methods for treating potential confounding factors (CF) in our test of the Linear-No Threshold theory with data on lung cancer mortality vs radon exposure for 1601 U.S. counties are reviewed. The basic treatment is stratifying the data on a CF and analyzing each stratum separately; this greatly reduces the confounding effects. This has been done for over 100 potential CF of various types -- socioeconomic, environmental, geographic, etc.. A method of correlation by rank with both radon and lung cancer is introduced as a rapid screening procedure; it has been used on over 500 potential CF. The decisive issue here is “plausibility of correlation” (PoC). For smoking, a CF that appears explicitly in the relationship being tested, correlations (not by rank) and width of the distribution are relevant. Poc is a very important tool here. Systematic differences in radon exposure for smokers and non-smokers is treated and shown not to be effective. Variations in smoking intensity (cigarettes per day) correlated with radon exposures are treated using an approach proposed by BEIR-VI, and even extended to include correlations with fraction of population that smokes. Again, PoC is the vital factor. Other issues treated include urban-rural differences in both smoking (urban people smoke more) and radon exposure (lower in urban homes), systematic differences in time spent in homes with high and low radon levels, combinations of CF, and time period in which lung cancer mortality is considered. In all of these studies, nothing was encountered that could substantially modify the original conclusion that Linear-No Threshold theory fails very badly in the low dose region, grossly overestimating the cancer risk from low level radiation.

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The Bolton-Brush Radiographic Growth Studies

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Preliminary results of a review of the current health status of subjects in a longitudinal radiographic study of human growth and development. The study was begun in the late 1920's and was focused on "normal" children from preschool years through teenage to early adulthood.

There were a total of 4300 persons associated with the investigation, with a range from a single series of radiographs to others with up to 12 complete series. Some individuals had as many as 45 pairs of cephalometric x-rays over the span of the active records accumulation. The collection totals approximately 250,000 radiographs.

Many of the subjects have been recalled over the last 10 years, giving a general picture of inordinately good health for their ages, of middle sixties through late seventies.

The series of diagnostic radiographs included all of the developing epiphyses of the body as well as a pair of cephalometric x-rays – a total of 13 to 15 x-rays at each examination.

The original directors of the associated studies, Dr. T.W. Todd of the Brush Inquiry, and Dr. B.H. Broadbent of the Bolton Study, exercised particular care in documenting the equipment, materials and x-ray exposures for each examination providing accurate data for reconstruction of the dosages involved.

The authors believe that the significant amounts of low-level radiation received by the subjects have indeed had no detrimental effect but rather a hormetic effect on the participants.

A visual history of the Studies' activities, participants and data reconstruction are presented.

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Effect of Low Dose Cadmium on Stress Proteins and Survival in Human Prostate Cells

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Cadmium is an environmental pollutant linked to prostate and lung cancers and is classified as a human carcinogen. Exposure to low doses of toxins such as cadmium can generate adaptive response, a phenomenon known as hormesis. Recent experiments with low dose exposures with cadmium suggest that cellular responses to these low levels may induce tolerance to cadmium and prove protective to higher toxic exposures. Cadmium has been shown to induce the expression of stress proteins, metallothionein (MT) and heat shock protein (HSP-70). This study evaluates the effect of low and ultra low doses of cadmium in normal human prostate cells (RWPE-1). These normal human prostate cells were treated with low and ultra low doses of cadmium for 20 weeks. No significant changes were detected in cell morphology, cell viability, cytotoxicity in these cells. However, a slight increase in metallothionein protein level was observed with low level cadmium treatments compared to normal cells. Gene expression patterns for MT and HSP-70 and the potential protective role of low dose treatments in these cells will be discussed.

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Non-Immunological Sensitization: A Nonlinear Host Dose-Response to Repeated Low Level Chemical Exposures

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An extensive body of pharmacological and biobehavioral research has demonstrated that subsets of animals and people exhibit a progressive amplification of response size over the course of repeated, intermittent exposures to the same dose of a given chemical or environmental stressor (Antelman 1994; Bell et al. 1992; Bell et al. 1999, 2001). Agents that can initiate sensitization include a wide range of structurally unrelated substances, e.g., volatile organic compounds such as formaldehyde or toluene, pesticides such as lindane, and stimulant drugs such as cocaine or amphetamine. Substances or stressors not involved in the initiation phase can then cross-sensitize and subsequently elicit amplified responses. Individual difference traits and factors that favor enhanced sensitizability include female gender, certain genetic strains (e.g., alcohol-preferring), sucrose preference, lateral asymmetry, as well as prenatal maternal stress or environmental impoverishment in early development. Brain mesolimbic dopaminergic pathways may mediate elements of the sensitization process. At physiological limits of a system, the direction of the sensitization can reverse with continued, repeated re-exposures, i.e., oscillation (Antelman and Caggiula 1996). Sensitization is a proposed model for a range of clinical disorders, such as craving in substance abuse, posttraumatic stress disorder, recurrent affective disorders, multiple chemical sensitivity syndrome, fibromyalgia, “Gulf War Syndrome,” and temporal lobe epilepsy. Our laboratory has shown sensitization of electroencephalographic and cardiovascular physiological parameters over multiple sessions in human subjects who report distressing symptoms from low levels of common environmental chemicals (representing approximately 15-30% of the general population), in comparison with controls who deny such illness from low-level exposures. This presentation will provide a summary of past sensitization research and recent studies on human chemical sensitization. The concept of individual differences in host susceptibility, with nonlinear responses to environmental factors, has significant implications for evaluation of hormesis-like dose-response patterns in living systems, notably in persons with various clinical conditions.

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Ultra-low Doses and Biological Responses: A Review and Recent Experiments

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Low-dose stimulation of recovery and repair processes are common biological phenomena leading to tolerance and growth stimulation induced by up-regulation of stress proteins, DNA repair enzymes and other cellular resistance pathways. There are a least two areas that have not been explored about these processes. One is whether these adaptive responses occur at doses lower than 2-10 logs below the No Adverse Effect Level (NOAEL) and the second is whether hormetic effects have utility in disease prevention or treatment. The alternative medical practice called homeopathy claims that both of these effects occur, but scientific evidence to support those claims is lacking. We agree there is no current scientific foundation for homeopathy, but believe that exploring the effects and utility of ultra-low doses using laboratory models is valuable. This presentation will present a critical review of current laboratory research on low and ultra-low dose toxicology research. We will also present recent experiments examining the stimulatory and protective effects of low and ultra-low dose neurotoxins, including glutamate, in cell and animal models. In cellular models of glutamate toxicity, we have found that both glutamate and cyclohexamide will protect against toxicity from exposure to toxic doses of glutamate. In addition, ultra-low doses of glutamate reduce brain damage in a stroke model by 40-50%. Other neurotoxins, such as Con-G, PLA2 and MPP+ do not afford protection. Data on KCL and NMDA is mixed. In addition, several heavy metals appear to be useful for induction of protection at low and ultra-low doses. Outcomes of research with mercury, arsenic and cadmium include accelerated clearance of the toxin, reduced mortality from lethal doses and prolonged up regulation of metallothionein (MT).. Further research on the potential use of adaptive responses to low-dose and ultra-low doss exposures is warranted.

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High Sensitivity ^{1}H-NMR Studies of Homeopathic Remedies: Unexplained Peaks in the Spectra of some Samples

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PURPOSE: To investigate the hypothesis that homeopathic remedies prepared in water might be distinguishable from control samples via high-sensitivity NMR spectroscopy. METHODS: Several series of the homeopathic remedies *Nat Mur* and *Arg Nitr* were made in water by Hahnemann's method, up to the 12C potencies, starting with 1 M and 0.1 M solutions of NaCl and $AgNO_3$ respectively. Controls were unprepared water (H_2O-u) and succussed water (H_2O-s). Samples were analyzed via ^{1}H-NMR using presaturation of the water peak in order to improve the sensitivity for signals away from the water peak. In all runs, D_2O was added for locking, acetone at 1:350,000 v/v was added as a marker, temp was clamped at 20°C, and at least 128 transients were taken. RESULTS: Zero of 6 H_2O-u samples had unexplained peaks, but unexplained peaks were seen at 1.74, 3.02, 3.27, and 8.27 ppm, in multiple remedy samples and in some H_2O-s samples. 17 of 29 samples that were NaCl or $AgNO_3$ potencies (all 6C and above) had the 1.74 ppm peak, and of these, 8 had the 8.27 ppm peak and 3 had the peaks at 3.02 and 3.27 ppm. Peak sizes ranged from 5 to 45 □m (by comparison with the acetone marker). There was no correlation between the remedy or potency and the signal pattern found, e.g., two samples of *Arg Nitr* 6C had no unexplained peaks, another had just the 1.74 peak, and a third had all four. Three of 5 H_2O-s samples had no unexplained peaks, one had just the 1.74 ppm signal, and one had all four peaks. These peak patterns do not resemble those of any common organic contaminant. Dissolved silica as an explanation was ruled out by a series of *Arg Nitr* potencies made in plastic tubs: some of these also showed the same peaks. CONCLUSIONS: (1) 19 of 34 samples that were remedies or H_2O-s had an unexplained peak at 1.74 ppm, and 9 had additional peaks at one or more of 3.02, 3.27, and 8.27 ppm; (2) 0 of 6 controls had unexplained peaks ($p<.04$); (3) there was no consistent signal pattern seen for a given remedy and potency; (4) the unexplained peaks do not match any common organic contaminant and do not come from the glass or plastic containers used. (5) These data support the hypothesis that some remedies and some H_2O-s samples are distinguishable via high-sensitivity NMR studies from H_2O-u.

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Challenges to the Investigation of Low and Ultra-low Dose Effects

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The basic research program on “homeopathy and self-healing” aims to understand (a) self-healing of cells after stress and damage at cellular level, (b) the homeopathic similia principle in relation to cellular self-healing program, and (c) the regulatory principles with respect to the influence of low and ultra-low doses in self healing.

A description of damage and self-healing at molecular and biochemical level has allowed a description of the essential homeopathic pillars (the pathogenetic trial, the remedy-symptom matching, and the treatment according to the similia principle) in molecular terms. A second type of descriptive studies on damage and self-healing, including biophysical points of view with respect to domain coherence and photon emission has allowed the construction of a general parameter for the characterization of early disturbances and recovery of a biological system.

The studies on the self-healing and similia principle can be characterized as straightforward studies. These studies are at the stage that they can be rather easily extended to a biological system with higher complexity that should allow the study of specific organ damage and recovery after exposure to certain types of intoxication.

In contrast, the studies on low and ultra-low doses were found to be more diffuse and time-consuming. In particular our studies on ultra-low dose effect in different biological models did not lead to reliable protocols.
Recently, we made new progress in this field. We followed a more systematic approach starting from the non-linearity of dose response curves in mildly damaged biological organisms. We have executed experiments that demonstrate that the cellular response depends on the severity of the diseased state, and that this is changing in time during the process of recovery. Since a stimulation of self-healing by low doses is expected only in cells that have not a maximal self-healing activity, this suggested that another protocol is required for this type of studies. A protocol that measures effects of a whole population of cells including non-responding, positive and negative responding cells is not desirable. In contrast, this other protocol must allow time series analysis of individual cells, where the diseased state can be registered non-invasively and the treatment can be adapted to the cell’s diagnosis. We have carried out systematic experimental and theoretical studies and selected a suitable biological model. New protocol and measurement techniques have been developed for a study on the effects of low and ultra-low doses. By combining the basic homeopathy research at the Utrecht University and the theoretical and technical experiences of the International Institute of Biophysics we expect this new research program on low and ultra-low dose effects can be carried out successfully.

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Low Dose Effects in Pulmonary Disease

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Variability in pulmonary dose response relationships clearly exist, both among practically healthy people, and between patients with recognized respiratory diseases. What has been puzzling to many clinicians and researchers is the report by some patients of adverse effects following low dose exposures. A striking characteristic of patients meeting a research definition for Multiple Chemical Sensitivity(MCS) is that subjective reports of distress far exceed the degree of impairment measured using traditional tests of respiratory function. This presentation will update evidence supporting the hypothesis that these patients have a disorder of the c-fiber nervous system with proximal respiratory epithelial dysfunction (typically squamous metaplasia) and plasticity of the nodose and jugular ganglia resulting in abnormal central processing of mechanoceptor stimuli and autonomic dysregulation.

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Non-linear Functions between Stress Hormones, Brain Plasticity and Memory

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Non-linear relationships between arousal level, or stress, and performance in behavioral tasks have been described for almost a century. Studies have shown that an intermediate level of arousal typically produces optimal performance, and either very low or high levels of arousal are associated with poor performance. My group has investigated the biological basis of this non-linear relationship between stress and brain processing in rats. More specifically, we have studied the relationship between corticosterone (a hormone released in response to stress) and both memory and the physiological processes which underlie memory (brain plasticity).

The first indication of a non-linear relationship between hormones and brain plasticity was our observation of a U-shaped relationship between the level of peripheral corticosterone and the magnitude of primed burst potentiation (PBP). PBP is a long-lasting form of brain plasticity produced by brief (< 1 sec) electrical stimulation of brain connections. The non-linear function between corticosterone and PBP suggests that brain processing is optimal under conditions in which there is an intermediate level of stress. Recently, we have made comparable observations in studies of rat memory under stress. We have found that rats with intermediate levels of corticosterone exhibited excellent memory. Shifting the animals away from the optimal range of corticosterone, produced by either stress or drug manipulations, resulted in memory impairments. The findings provide insight into how stress affects memory, and also have implications toward understanding how prescription forms of corticosterone, e.g., cortisone or prednisone, may affect brain and behavior in people. Thus, intermediate doses of cortisone or prednisone can enhance cognition, but high doses of these steroids can interfere with memory by interfering with brain plasticity. In summary, this work indicates that optimal memory and brain processing occur under conditions of intermediate arousal, and reduced capacity occurs under very low or high levels of arousal.

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Biphasic Effects of Progesterone Treatment on Proliferation of Normal and Malignant Human Ovarian Surface Epithelial Cells

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Progesterone has been used in the clinical treatment of ovarian cancer (OCa) and has been shown to reduce the risk of developing ovarian cancer in premenopausal women who have undergone hormonal replacement therapy, which contain estrogen and a high dose of progesterone. However, the mechanism of anticancer effects by progesterone has not been understood. In this study, the effects of progesterone on primary cultures of human ovarian surface epithelial (HOSE) cells, nontumorigenic HOSE cells and in OCa cell lines were investigated. After the incubation with increasing concentrations of progesterone, the viability of the cells was evaluated by MTT assay. Interestingly, progesterone (P4), at low concentrations (10(-11) to 10(-10) M), was stimulatory to HOSE and OCa cell growth, but at high doses (10(-8) to 10(-6) M), P4 exerted marked inhibitory effects. Annexin V staining revealed early apoptotic events in these cells 6 hours after treatment of cells with highest 10(-6) M concentration of progesterone.To better understand both proliferation-inducing and -inhibiting effects of progrsterone, two isoforms of progesterone receptor, the 120-kDa B receptors and the N-terminally truncated 94-kDa A receptors, that have unequal transcriptional activities, differentially regulated by hormone treatments, are presently under investigation. In conclusion, contrary to low concentrations, higher concentration of progesterone inhibits the proliferation and elicits apoptosis of HOSE and OCa cell growth. These results demonstrate that progesterone at a concentration similar to that seen during the third trimester of pregnancy exhibited a strong antiproliferative effect on primary, immortalized and ovarian cancer cell lines.

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Two Examples of Paradoxical Pharmacology Using *In Vivo* Animal Models of Disease

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The possibility of using drugs currently contraindicated for a disease to treat that disease has been the subject of a recent article (Bond, Trends Pharmacol. Sci. (2001) 22, 273-276. The basic premise being that acute and chronic effects of drugs can often be opposite in nature, yet the contraindication is often based on the acute effect. For example, ß-adrenoceptor antagonists (ß-blockers) were originally contraindicated is congestive heart failure (CHF). However, chronic treatment with these drugs produces a paradoxical decrease in mortality. *In vivo* models of two disease states have been studied to test the hypothesis of paradoxical pharmacology. The first was an allergen-challenged mouse model of asthma. Sensitized/challenged tracheal tissue was significantly more responsive to the spasmogen methacholine vs sensitized/nonchallenged trachea. (946.4 ± 227.7 mg vs. 537.5 ± 122.7 mg, respectively (n=7; $P < 0.05$, ANOVA). The spasmogen response to methacholine from mice treated with carvedilol or alprenolol for 18 days was significantly reduced from that of sensitized/challenged or sensitized/nonchallenged tissue (258.9 ± 84.0 mg and 162.5 ± 42.5 mg, respectively (n=7-8, $P < 0.001$, ANOVA). Also, guinea pigs were sensitized, treated for 28 days with carvedilol, and tested for a wheal and flare response to subdermal ovalbumin; 88% control animals had a positive response; however, 0% of guinea pigs treated with carvedilol reacted positively to the ovalbumin. The second was a rat model of hypertension. We infused spontaneously hypertensive (SHR) rats (8 week old) with Norepinephrine/Epinephrine (NE/E) (150 ng/kg/min i.v.) for 2hr/d for 27 days using osmotic minipumps connected to a Lynch coil cathether. Blood pressure (BP) readings were taken daily outside the infusion window. The group treated with NE/E showed a significant drop in BP 48 hrs after the last dose (-54±7.8 mmHg vs –13±4.2 mmHg for NE/E and vehicle, respectively. n=3; $P<0.001$, ANOVA). In addition, 48 hrs after the last dose, we infused the nitric oxide inhibitor L-NAME (10 mg/kg i.v. for 10min) and measured BP before and after infusion. The NE/E treated SHR rats had an increased pressor response to L-NAME infusion vs SHR rats treated with vehicle (66±1.4 mmHg vs to 33±1.8 mmHg for NE/E and vehicle groups, respectively, n=2). Based on the results from these two models, paradoxical pharmacology may be beneficial. This hypothesis needs to be further tested in other disease states.

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Using *C. elegans* to Model Induced Stress Resistance and Life Span Hormesis

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The nematode *Caenorhabditis elegans* has been widely used in genetic studies. We have applied this convenient and powerful model organism to the study of hormesis. *C. elegans* displays induced resistance or nonlinear responses to a variety of stressors, including heat, oxygen, and a chemical reactive oxygen species (juglone). The observed hormetic benefits are not limited to subsequent resistance to the same stressor, but include resistance to other stressors (cross-tolerance) as well as increased life span. We have found that the hormetic life extension requires the function of several genes of the insulin/IGF response pathway. In the nematode this pathway was originally identified by its role in the formation of dauers, a stress resistant and long-lived alternative larval form. Although greater stress resistance and life extension are thought to be intimately connected, we found that the genes required for hormetic life extension are not all critical for the induction of robust thermotolerance observed in response to the same pretreatment. This raises the possibility of a partial genetic separation of induced stress resistance and induced life extension in *C. elegans* and may shed light on the overall aging process.

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Biological Aging and its Hormetic Modulation by Repeated Challenge

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Biogerontology – the study of the biological basis of aging – has now developed a solid scientific base with respect to the understanding of the phenomenon of aging. New and innovative strategies and approaches are being increasingly applied to modulate the process of aging. One of the major concepts emerging from research in biogerontology is that of aging as a failure of maintenance. The ability of living systems to respond to internal and external sources of damage, such as free radicals, ultraviolet radiation, heavy metals, toxins and thermal stress, is an indicator of their property of homeodynamics. It is by virtue of this ability that they counteract stress and adapt for continued survival. Therefore, one approach is to use repeated challenge as a stimulator of maintenance and repair pathways resulting in the modulation of the aging process. In a series of experimental studies we have shown that repetitive mild heat stress has anti-aging effects on growth and various other cellular and biochemical characteristics of human skin fibroblasts undergoing aging in vitro. We are characterising the hormetic effects of repeated challenge at the levels of maintenance of stress protein profile, reduction in the accumulation of damaged proteins, stimulation of the proteasomal machinery of protein degradation, and maintenance of differentiated cell functions during cellular aging. This strategy of hormetic challenge is a method to improve and maintain the biochemical and physiological performance of cells, tissues, organs and organisms. The ultimate aim of such studies is to develop practical means to prevent or minimise the age-related impairments resulting in the onset of diseases and to for living a healthy old age.

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Hormetic vs. Inhibitory Effects in Sea Urchin Bioassays

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A two decade-long experience has been established in testing the effects of a broad range of xenobiotics and of physical agents in sea urchin early development. This experience has led to the observation of a number of different effects both including inhibitory and hormetic effects towards developmental, reproductive, genetic and biochemical endpoints in sea urchin bioassays. This bioassay model allows to evaluate several parameters including changes in: a) fertilization success; b) larval differentiation; c) mitotic abnormalities; d) transmissible damage from to gametes to offspring, and e) biochemical activities. While toxic outcomes have been invariably included in published reports, the observation of hormetic effects has been as yet confined to fertilization success and biochemical markers. This apparent inconsistence may be explained by the current criteria in control quality for developmental defects and mitotic abnormalities, leading to the exclusion of an embryo lot when controls are considered inadequate because of excess abnormalities. The spermiotoxicity and biochemical tests are better suited for the observation of either hormetic or inhibitory effects. When testing fertilization success, sperm:egg ratio and duration of sperm pretreatment are designed in order to obtain a “suboptimal” fertilization rate in control schedule ranging 70 to 50%, thus allowing the observation of both spermiotoxic and hormetic effects as a result of sperm exposure to increasing agent levels. An analogous sensitivity is achieved in biochemical determinations, where control levels are not assigned a priori, thus allowing any positive or negative shifts from control values to be measured. A set non-monotonic dose-related trends following exposures to chemicals, mixtures and gamma radiation is reported to substantiate the previous experience.

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Non-Linear Factors Affecting Exposure and Risk to Anthrax

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Although anthrax is a rather commonly-occurring, globally-distributed disease of humans and livestock, its use as a bioterrorism agent in the U.S. mail last Fall has lead to renewed interest in the basic mechanisms of this disease. Naturally-occurring inhalation anthrax has a relatively low rate of infectivity, with an infectious dose commonly reported to range from 8,000 - 10,000 spores, with some reports as high as 50,000 spores. However, laboratory-grown and purified anthrax, sometimes referred to as “militarized” or “weaponized” anthrax, has been reported to have a higher rate of infectivity (require fewer spores to cause infection) than naturally-occuring anthrax. Although it may seem intuitive that purified anthrax would logically be more infective, this would not explain differences in infectivity on an exposure-dose basis, that is, on the basis of absolute number of spores. It is theorized that these differences may be due to varying and inconsistent use of the terms *spore, particle,* and *infective dose,* as well as to differences in the alveolar deposition of anthrax particles of varying sizes on a fractional (total mass) basis, differences in alveolar deposition of anthrax particles of varying size on a relative number basis, differences in number of anthrax spores per particle size, and differences in number of spores delivered to pulmonary macrophages on a relative particle-size basis.

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Biphasic Effects of Cardiac Glycosides (Ouabain) on Vascular Smooth Muscle Cell Proliferation

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Cardiac glycosides are well known inhibitors of membrane Na, K pumps, and have been used for centuries as therapy for cardiac failure, since they increase myocardial contractility via inhibition of the pump. The therapeutic effect occurs by a pump related increase in Na_i and subsequent reversal of the Na/Ca exchanger. In addition, it also has been shown that compounds similar if not identical to these glycosides have been isolated from a variety of mammalian tissues. There have also been suggestions that these endogenous compounds (endogenous digitalis like factors, or EDLF) may be involved in certain types of hypertension.

We have shown that low levels of ouabain, a relatively water soluble glycoside, can activate proliferation of canine vascular smooth muscle cells at concentrations below those which affect cellular ionic content (0.1-1.0 nM) i.e. no pump inhibition. Higher concentrations of the drug (10 nM) have no proliferative effect, and actually, inhibit cell proliferation when compared to control. Additionally, we have shown an identical effect on a rat vascular smooth muscle cell line (A_7R_5) which occurs at drug concentrations three orders of magnitude higher than with canine cells. Since this difference reflects the affinities of ouabain for the canine and rat pumps respectively, the data strongly suggest the drug is binding to the α subunit of the Na pump, rather than to another as yet unidentified protein. The mechanism of proliferative activation involves activation of ERK ½, via src and transactivation of the EGF receptor, while inhibition may involve apoptosis.

This is the first time that cardiac glycosides have been demonstrated to have specific physiological effects at concentrations below those which inhibit the Na K pump.
Thus, relating our data to clinical situations, there appear to be potentially three levels of glycoside effects, manifested as: 1) no pump inhibition, (proliferation); 2) “physiological” pump inhibition (positive inotropy) and 3) “pathological” pump inhibition, (toxicity).

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Altered Phenotype in Glial Cells Underlies the Low-dose Neuroprotection against Neurotoxicity

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Nervous tissue contains a range of protective systems against the damaging effects of toxic chemicals. High levels of the protective systems are localised in the astrocytes. Both in situ and in vitro astrocytes respond to low doses of toxic substances with alterations in phenotype termed astrocyte activation. The alterations affect a range of structural and functional properties of the cells, with the upregulation of a large number of molecules, including those controlling the protective systems. Experiments with cultures of primary rodent astrocytes and glioma cell lines show that toxicity markers (including the structural protein GFAP, components of energy metabolism and antioxidant protective systems) frequently respond in bi-phasic patterns, with the characteristics of a hormetic dose-response response relationship. The responses are caused by toxic substances with widely different structures and mechanisms of action. The early phase, associated with increased protection, can in turn be correlated with increases in antioxidant protective systems and other components of the altered phenotype, for example the cell hypertrophy with increased GFAP. It is concluded that astrocytes, activated by relatively low doses of toxicants, may provide enhanced protection of the nervous tissue against damage.

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Is the Hygiene Hypothesis an Example of Hormesis?

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Over the last 30-40 years there has been a steady worldwide increase in the prevalence of allergy and asthma among children, especially children in developed countries. One explanation that has been proposed for this phenomenon is the "hygiene hypothesis", which states that microbial exposure early in life may be needed to stimulate the transition from an immature immune system that is predisposed to allergy to a more adult immune response. Under this hypothesis, vaccination, antibiotic use, and the increasingly "hygienic" environment of modern life have resulted in decreased early-life infections and microbial exposures and subsequently caused an increasing predisposition for the development of allergic disease. In some respects, this concept of beneficial effects (i.e. immune stimulation) from exposures that have generally been perceived as harmful (i.e. microbial agents) suggests hormesis. We will address this concept, focusing on three general aspects of the hygiene hypothesis:

1. The possible beneficial effect of infectious illness in early childhood,
2. The possible beneficial effect of early life exposure to ambient microbials from "dirty" environments,
3. The possible beneficial effects of subclinical infections.

Each of these aspects will be discussed and evaluated to see whether a hormetic mechanism might apply.

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Tissue-Specific Dysfunction Induced by Menadione in Blood Vessels: Mechanisms for U-shape Dose-Response Curve

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Although several studies have shown that treatment with menadione leads to endothelial cell cytotoxicity, investigations of menadione's effects on blood vessels are limited. To determine if menadione affects vascular function, we investigated the effect of menadione on blood vessels using the isolated rat aortic rings in *in vitro* organ bath system. Treatment with menadione revealed the U-shape dose-response curve and thus, two independent mechanisms over a wide range of concentrations were examined in menadione-induced vascular dysfunction. Menadione (1-10 μM) potentiated phenylephrine- and serotonin-induced vasoconstrictions in aortic rings with endothelium. Menadione at these concentrations induced oxidative stress generating superoxide anions and inhibits endothelial nitric oxide synthase (eNOS), which in turn causes inhibition of nitric oxide pathway and subsequent endothelial dysfunction. On the other hand, higher concentrations of menadione (> 10 μM) inhibited phenylephrine- and serotonin-induced vasoconstrictions in aortic rings with endothelium and treatment with 50 μM menadione for 30 min abolished agonist-induced vasoconstriction completely. These inhibitory effects were seen in aortic rings without endothelium, suggesting that menadione at higher concentrations may interfere the contraction machinery in vascular smooth muscle. Consistent with these findings, treatment with menadione to aortic rings without endothelium resulted in dose-dependent inhibitions of intracellular calcium level and myosin light chain phosphorylation induced by agonist. Unlike lower concentrations of menadione, hydrogen peroxide may play an important role in smooth muscle dysfunction, since treatment with catalase reversed the inhibitory effects of menadione on agonist-induced vasoconstriction. These biphasic effects by menadione were irreversible and not due to its cytotoxicity (assessed by LDH leakage). Collectively, menadione, and perhaps other quinones, can alter vasomotor tone in a U-shape pattern through tissue-specific dysfunction that may increase risk for development of vascular diseases. These results will also provide the new insights into the mechanisms of tissue-specific dysfunction induced by menadione in blood vessels.

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Non-Linear Dose Response: Legal Standards for the Admission of Novel Scientific Theories in Regulatory Decision-making

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Regulatory agencies have traditionally assumed a linear, non-threshold (LNT) model for assessing the risks of hazardous agents. In recent years, agencies have been pushed to depart from this LNT assumption by new scientific findings. Examples include scientific findings on toxicological thresholds, hormesis, non-genotoxic carcinogens, and endocrine disruptors, all of which suggest potentially non-linear dose -response relationships. The legal standards governing the scientific sufficiency and reliability necessary to incorporate such novel scientific theories into regulatory decisionmaking is examined from both an empirical and normative perspective. First, the consistency and key factors involved in agency decisions to accept or reject novel scientific theories are assessed. Relevant factors include the quantum and type of scientific evidence supporting the novel theory, the extent to which the theory has been endorsed by expert scientific groups, whether or not a mechanism for the new theory has been demonstrated, and the consequences of the new theory on the regulatory outcome. Second, potential legal standards that should govern the admission of novel scientific theories are discussed. The legal system has yet to impose a systematic, consistent set of principles governing the incorporation of new scientific evidence into regulatory decisions. Rather, such decisions have been made largely on a case-by-case approach. In contrast, in product liability and other common law cases the Supreme Court has imposed in its *Daubert* decision a standard for the admission of scientific evidence that evaluates both the relevance and reliability of the scientific evidence. The utility, feasibility and content of a similar admissibility framework for the admission of scientific evidence in regulatory decisions are evaluated, using non-linear dose-response relationships as a case study.

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Implications of Hormesis for Industrial Hygiene

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This paper considers hormesis as a valid and potentially valuable alternative hypothesis for low-dose response in the context of occupational health risk assessment. It outlines the current occupational risk assessment paradigm and its use of high-dose toxicological data in setting occupational exposure limits (OELs). This present effort is a call to science to investigate the potential promise of hormesis in providing a *prima fascia* experimental evidence for a low-dose threshold of toxic effect to chemical agents. The scientific effort and advancement advised in this piece could also lead to experimentally validated quantitative estimates of the toxic effect extant at occupational exposures in the region of the OEL.

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Do we Need Low Residential Radon Limits?

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The beneficial and detrimental effects of radon are the oldest known and most widely studied radiation effects on man. A multitude of dosimetric, epidemiological, and other radon studies still continues not only because it is easy to measure, but because of its large contribution to natural population exposures. More importantly, with its wide variations in natural occurrence, it may also be a crucial test for the currently dominating LNT hypothesis. As a result of international recommendations such, e.g. by IAEA and BEIR VI, assuming an increase in the lung cancer risk of about 10 % for 100 Bq/m³, EPA recommends a 140 Bq/m³ limit, and the EU countries consider regulations for intervention levels of 200 Bq/m³ for new and 400 for old residential buildings, and somewhat higher levels for working places. However, not only U.S. citizens are reluctant to accept radon reduction measures despite various "educational" activities by the authorities. This would involve expenses and inconveniences, and they are not convinced that they lived up to now with a "health hazard".

Most Europeans are also aware of the fact that there are many medical radon therapy facilities, e.g. 12 in Germany and Austria, in which ca. 75.000 patients annually are treated for rheumatic and arthritic problems, Morbus Bechterew, and other diseases. In recent years, several careful randomized double-blind medical studies confirmed the pain-reducing and other beneficial effects of radon balneology up to at least six months after the treatment, even if the mechanism of such effects (empirically known in several European cultures and Japan for hundreds of years) is not yet fully understood.

An increasing number of radon specialists also has serious doubts about the validity of various, in some cases rather expensive epidemiological studies on residential radon health effects, and the debate continues about the merits and problems of different methods (such as ecological, cohort, and case-control studies), and the correct interpretation of the results in the region below about 500 Bq/m³. So far, such studies did *not* produce any evidence of detrimental health effects in this region. Nevertheless, the recommendations and regulations are based on the linearity (LNT) hypothesis by extrapolating linearly from very high miner exposure levels under completely different conditions down to zero, frequently without adequate consideration of dominating confounders such as smoking, uncertainties in retrospective dosimetry, etc. A meta-analysis or "pooled" data, as currently in progress in the U. K. and Canada, will *not* be able to overcome such bias, as in particular the determination of past smoking habits will remain highly uncertain. Studies with confirmed never-smokers may help to solve this problem, even if the case numbers are much smaller.

This situation raises interesting questions not only about recent residential and working-place radon exposure regulations in and outside Europe, but also regarding the validity of the LNT hypothesis and the closely related Collective Dose Concept; the cost/benefit assessment of efforts to reduce very low exposure levels far below the fluctuations of natural exposures in remediation, decommissioning, and radioactive waste programs; and their social, economical and ethical impact. Some of these issues will be briefly discussed.

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Some Thoughts About How To Incorporate Hormesis into the Risk Assessment Process

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Given that hormetic effects have been observed with a wide variety of chemicals both *in vitro* with mammalian cell lines and *in vivo* with laboratory animals, it is reasonable to suggest that such effects might occur in humans exposed to low levels of environmental and/or dietary contaminants. If true, then there would be some significant implications to the human health risk assessment process. Currently, most regulatory decisions regarding chemicals in the environment are driven by risk assessments in which the estimated exposures are very low, yet because of the manner in which the risks are estimated, the risks are often considered significant. For example, the EPA's "reference dose", which is used to characterize the hazards of noncarcinogenic chemicals in the environment, is typically set at levels several-fold below the NOAEL observed in animal studies. Also, because the current EPA paradigm dictates that all carcinogens must be assumed to have no threshold, low doses of environmental carcinogens that may in fact be "health protective" (hormetic) are still assumed to pose a cancer risk, and possibly a significant one that requires regulatory action. We propose a preliminary research program in which existing data on hormetic effects could be factored into the risk assessment decision-making process. First, a set of minimum response criteria should be established which, if met, would be taken as sufficient weight-of-evidence of the possible existence of hormesis. Second, the toxicology and epidemiology literature should be reviewed for evidence of hormesis for the approximately 20-30 chemicals which tend to be of most environmental concern: arsenic, lead, cadmium, DDT, PCBs, PCDD/Fs, trichloroethylene, and others. Then, for those chemicals for which there is sufficient evidence to suggest that there might be health-protective effects at low doses, a rough approximation of a "hormetic dose range" should be established, if possible. A comparison of the estimated background (e.g., dietary) and environmental doses of these chemicals to their respective hormetic dose range would become a standard component of the risk characterization process. A comparison demonstrating that the estimated dose is at or below the hormetic dose range might influence regulatory action (regarding proposed environmental cleanup, protection of the food supply, etc). In addition, the EPA toxicity criteria (reference doses [RfDs] for noncarcinogens and risk-specific doses [RSDs] for carcinogens) for these chemicals should be compared to the hormetic dose range in all risk characterizations If these criteria fall within or below the range, then some modifying factors should be considered in the environmental risk assessment process. Ultimately, it may make sense to adjust certain RfDs and RSDs upwards.

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Radiation Hormesis: Molecular-Cellular Biology, Epidemiology, and Prevention and Therapy of Cancer

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Aging, mortality, and cancer mortality are generally accepted to be associated with stem cell accumulation of permanent alterations of DNA ("mutations"). Free radicals of oxygen initiate reactive oxygen species (ROS) that induce DNA damage and signaling to cells and tissues with subsequent stimulatory responses. The antimutagenic DNA damage-control system is the central component of homeostatic control essential for survival. Over eons of time a complex biosystem evolved in aerobic organisms to control the vast number of DNA alterations (oxidative adducts) produced by ROS generated principally by leakage of free radicals from mitochondrial metabolism of oxygen. Genetic defects of this antimutagenic system increase cancer and other mortality at an early age. In humans about a billion free radicals per cell per day are derived from abour 0.25% of all metabolized oxygen. In a low background radiation area of 1 mGy/y, antioxidants and other intermediate reactions prevent all but one in a thousand of these ROS from altering DNA, resulting in about one million DNA alterations/cell/d. This initial damage is reduced to about 1 mutation/cell/d by enzymatic DNA repair and removal of persistent DNA alterations by apoptosis and the immune system. Production of mutations by endogenous oxygen metabolism is about 10 million times greater than mutations produced by low background ionizing radiation. Nevertheless, ionizing radiation has a very significant effect on DNA damage-control as the result of spatial and temporal differences in the DNA alterations it produces. High-dose, high-dose rate radiation suppresses the activity of each component of this homeostatic biosystem with consequent increased mutations and increased mortality and cancer mortality. Low-dose radiation, on the other hand, stimulates each component of the antimutagenic system that decreases metabolic mutations; thereby decreasing cancer mortality and increasing longevity. This biphasic reaction of antimutagenic responses to high and low doses of radiation, predictably precludes a linear dose-response relationship of radiation and health effects. A tenfold increase of background radiation from 1 mGy/y to 10 mGy/y stimulates overall DNA damage-control activity by about 20%, producing a corresponding decrease in metabolic mutations and associated decreases of cancer mortality and mortality from all causes. Radiation hormesis is the biological basis of statistically significant epidemiologic observations of increased longevity and decreased cancer mortality associated with low-dose radiation. Immune system destruction of cells with persistent DNA damage is an essential component of effective antimutagenic control of malignant cells and tumors. Low-dose stimulation of the immune system may not only prevent cancer by increased removal of premalignant or malignant cells, but may also destroy gross cancer tumors with metastses. These findings have been reported in mice for almost 40 years, more recently in rats and humans.

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1. Neurobehavioral Hormesis: The Exclusion of Compounds that Activate Arousal Systems in the Brain

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The novel concept of neurobehavioral hormesis has been proposed as a concept with utilitarian value for neurobehavioral toxicology. Based on limited early data, the working definition for neurobehavioral hormesis is, “Persisting improvement in neurobehavioral performance capacity following low level exposure to an agent that produces denigrated neurobehavioral performance at levels above the No Observable Adverse Effect Level (NOAEL).” Given this definition, we propose compounds whose direct effects include direct activation of arousal systems in the brain should not be considered cases of neurobehavioral hormesis. For instance, typical psychostimulants, such as amphetamine, cause increased arousal at all doses. A mildly heightened state of arousal is often beneficial and results in improved performance in many neurobehavioral tasks. In contrast, at the higher doses the over-arousal is such that task performance begins to degrade. Such “inverted-U” dose response effects are best explained by the Yerkes-Dodson Law which is specific for different states of arousal. Currently, we propose to exclude such effects for two major reasons. Foremost, the effect exists only for as long as the exposure. The behavioral manifestations do not persist after the compound has been cleared from the system. Furthermore, traditional hormetic effects are opposite to the expected effect of the exposure. In the case of psychostimulants, the expected effect of heightened arousal is the cause of both the improved and the denigrated performance. One example of an hormetic effect that is not directly attributable to central nervous system arousal has been observed in animals exposed to jet fuel. At high doses, deficits in performance on a complex operant learning task were observed. In contrast, performance was modestly improved in the low dose group. Under the current definition, such effects qualify as hormetic, and may serve as an example of what may prove to be a relatively prevalent phenomenon of neurobehavioral hormesis.

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2. Debates Over Linearity: Much Ado about Evidence; Not Enough about Relevance nor Preference

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Any policy debate requires a weighing of facts and values. In the case of the regulatory debate over when to depart from low dose linearity, the relevance of the facts (evidence) has been overrated, and the role of values (societal preference) underrated. Current practice tends to infer dose-response shape from in-vitro (e.g., Ames test outcome) or in-vivo experiments (involving animal models, e.g., rat or mouse studies), without properly reflecting upon the limited relevance of this evidence to humans. It also tends to invoke a generic science-based notion of proof, thus overlooking societal preference about what balance to strike when weighing the costs of potentially making the wrong choice (misclassification costs). Using a stylized representation of the dose response dilemma, we demonstrate that the appropriate burden of proof is largely a function of societal values (preference). The chemical-specific marginal costs of control (the expense of precaution) is most important --- the marginal cost of residual risk is also important, though less variant across chemicals. Facts, such as the degree of sublinearity are surprisingly inconsequential. Of course evidence does play a role in assessing whether the burden of proof has been met. However, its' limited relevance (upon fully recognizing the contingencies of extrapolating shape across species, effect endpoints, individuals etc) may preclude exceeding the chemical specific burden of proof. In such cases, debates over the evidence are irrelevant. Future debates should be re-framed to consider the misclassification costs, and the limited informativeness of the available evidence (about low dose shape).

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3. Effects of Superlow Doses

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In 1983, we, at the Institute of Biochemical Physics, discovered that biological agents in concentrations from 10^{-3} to 10^{-14} and lower display the same activity (both qualitative and quantitative) as in concentrations from 10^{-4} to 10^{-5} M. Between these two extrema, there is the "dead zone": in this concentration range, there is either no effect or the effect is significantly weaker. It was concluded that biologically active substances (BAS) in concentrations much below NOEL (the level of preparation non-efficiency) can display a high biological activity. The studied BAS were natural antioxidants, regulatory peptides, antitumor compounds, adaptogens, neuromediators, herbicides, regulators of plant growth, antidepressants, nootropic preparations, and many others. For all the substances, a nonlinear dose—effect dependence was noted as characteristic. We attribute this dependence character to a change-over in the mechanism of action (or targets) with a change in the agent dose. The research results showed that the efficiencies of BAS superlow doses (SLD) and low-level physical factors are unique phenomena that are not associated with any particular chemical structures or biological organization levels. We formulated scientific principles of applications of BAS SLD and low-level physical factors as nontraditional methods of therapy. An important conclusion is the discovery of enhancement of sensitivity of biological objects to a variety of agents as a consequence of low-level effects produced, as well as elimination of toxic side-effects of medicinal preparations, addiction to drugs, etc. The studies conducted for people --- participants of liquidation of the ChPP accident who received low oses of ionizing irradiation --- showed a feasibility of tests aiming at determining individual sensitivities to impacts of low-level factors.

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4. New Approaches to Modeling of Living Systems Response to Ultra Weak Actions

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Response of living and physico-chemical systems on ultraweak action may not be revealed until adequate method of analysis is used. The specificity of living systems response is determined by background and subbackground action level, which is evolutionary customary. The system not recognizes this action as significance external one and has a power to compensate such “signal” by internal resources. Compensation is able to keep systems parameters inside a normal range. So, we may not notice any changes by means of standard descriptive statistics. But these small changes may become apparent in the interrelationship between variables, which may be revealed by correlation analysis and some integral measure of difference (multivariate distance). Experience shows, that it is true. The application of some integral index of correlation and multivariate distances allows discovering the effect of low-level radiation on individuals and people populations, for example. Chronic “signal” action may lead to changes not variables, but parameters of system and, hence, is able to move phase point to unstable region near bifurcation. Low stability near bifurcation point leads then to the increasing of system noise level. Measuring any flows, emitted by the system (electromagnetic, optic, thermal and so on), is a potent source of information about the system state. So, the methods of analysis of noise-similar processes, the revelation of hide and quasiperiodic patterns are really useful for evaluation of system state changes, as a result of action. Examples exists for application such a methods to cell populations and, even, to non-living physico-chemical systems.

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5. Effects of a 'Lead-free' Environment on Fertility and Reproductive Function in Female Mice

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Clinical and epidemiological investigations have provided conclusive evidence of lead-related impaired fecundity and reproductive function in women while in men [5-9], have yielded conflicting results regarding the effects on fertility.
In animal studies reduced fecundity and procreativity were generally reported in female mice esposed to high Pb exposure levels from diets while adverse effects were not consistently observed in male mice.

However, the effects of exposure to very low doses of lead on the aforementioned biological functions have yet to be investigated.

In the present study we assess the adverse effects of low levels of lead on fertility and reproductive function in female mice. The exposure groups of Swiss mice were: 4 ppm (Group I) and 2 ppm (Group II), rated as “very low exposures”; 0.2 ppm (Group III, control) and finally 0.02 ppm (Group IV) which we considered a “lead-free” environment.

Life-long lead exposure, which initiated in utero for all the mice, was continued until the animals were sacrificed.

Female 18-day-old offspring from all groups were divided into two subgroups, the first of which (subgroup A) was housed with sexually mature adult 3-month-old males, while the second one (subgroup B) with male siblings.

Fertility and reproductive function in females of subgroup A of Groups I - II no differences in the times of vaginal opening, oestrus, vaginal plug and first parturition when compared to those of subgroup A of the Group III. However, there was a significant reduction in the same times occurred in the females of subgroup A of Group 4 compared to the other groups. Blood estradiol levels confirmed these findings.

Fertility appears to be neither impaired nor enhanced at the same Pb exposure levels in male mice.

Furthermore, in animals living in group IV, red blood cells (RBC) were higher than in other groups, and we corrected PbB values to take into account the number of RBC with the result of a significantly lower PbB/RBC ratio in group IV compared to the other groups.

On the basis of our results we can hypothesize that fertility and reproductive function are enhanced in the female mice living in a “lead-free” environment, i.e. at Pb exposure levels below those found in the general environment and that are currently held to be “acceptable”. Lead consequently exerts a moderate inhibitory effect on hypophysary and gonadal functions even at very low doses.

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6. Pseudo-Hormesis: An Explanation in Search of a Manifestation

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Considerable evidence has accumulated that the biological activity of some agents is best described by low-dose non-linear dose-response relationships (e.g., "hormesis"). Whether such relationships are the rule or the exception remains uncertain. Non-linear dose-response has been generally attributed to peculiarities of biological response, with seemingly paradoxical response augmentation at low doses. Such observed effects, however, might be due instead to incorrectly chosen exposure metrics. For example, a biological response correctly characterized by low-dose linearity might appear non-linear if exposure were measured by a "biologically incorrect" metric not linearly related to the "biologically correct" metric. To explore this possibility, we reanalyzed a set of >1000 personal air samples obtained for exposure assessments of diesel exhaust in underground and surface mines. Samples were analyzed for carbonaceous carbon ("elemental carbon", EC), organic carbon (OC), and total carbon (TC). Across mines, arithmetic mean EC varied from 59-402 ug/m^3 underground and 2-6 ug/m^3 above ground, while arithmetic mean OC varied from 27-122 ug/m^3 underground and 28-92 ug/m^3 above ground. EC, OC and EC:OC ratio demonstrated independent non-linear relationships to TC and to each other. These findings have important implications for risk assessment of diesel exhaust. The two diesel risk assessments currently most influential assume linearized low-dose response curves, but used different exposure metrics: Steenland (1998) used EC, while Dawson and Alexeeff (2001) used TC. Toxicological data suggest that neither is "biologically correct". If there were such a correct metric with linearized low-dose characteristics (e.g., PAH components of OC), then sufficient epidemiological data would show that both EC and TC yield non-linear dose-response curves for lung cancer because both are non-linearly related to OC. Unfortunately, existing data are not yet sufficient to test this likely example of "Pseudo-Hormesis".

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7. Usefulness of Very Low-Doses of Cisplatin in Regulation of Animal and People Health Conditions

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In doses used for antitumor therapy, cisplatin is a very toxic substance which induces nephrotoxicosis, bone marrow injury with anemia, trombocytopenia and other adverse effects as blindness, deafness, severe nausea, vomiting, hearing loss and allergic reactions. Because of such high toxicity of cisplatin it was a great interest to examine whether low doses of this cytostatic drug can inhibit the tumor cell growth in vitro and whether long-term treatment of healthy animals with very low cisplatin concentrations can exert a toxic effect. In this aim the hematological and biochemical parameters in healthy rats and mice treated for two or six weeks with 0.5 ml of 10^{-8} and 10^{-16} mg/cm^3 of cisplatin solutions was measured in blood, liver and kidneys of animals. Parallel the condition of human lung carcinoma cells line A_{549} (ECACC No 86012804) after treating with the same doses of cisplatin was studied. The results of experiments strongly indicated that low doses of cisplatin can be stimulating for healthy cells but cytostatic for tumor cells. Based on these observations the solution of low doses of cisplatin was served to volunteer people during the chemotherapy courses. The index of tumor cells in blood diminished distinctly. Also the improvement of health conditions and psychological comfort were rapid and it seems that these opportunities could be useful in supplementation of cisplatin therapy. According to low of hormesis it was showed the decreasing of organism intoxication and increasing its' vitality under the influence of small doses of the same substance which had the toxic effect.

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8. The Effect of Low-Dose of Guaiacol on Enzymatic Activity of Fungal Cells

Elżbieta Deranalowicz-Malarczyk[1], Anna Jarosz-Wilkołazka[2], Janina Kochmańska-Rdest[2], M. Curie-Skłodowska University, Lublin, Poland.

In our lab we work with fungal material, which are cultivated in various conditions for their known possibilities of recalcitrant compounds degradation. Among them the artificial and natural activation of enzymes, such as laccase, peroxidases and catalase are in area of our interest. The objects of this study were selected strains of *Basidiomycetes* (*Pleurotus sajor-caju*, *Trametes versicolor, Abortiporus biennis, Cerrena unicolor*, and *Bjerkandera fumosa*). We tested the influence of guaiacol, the common and simple product of natural lignin and phenolic substances transformations, on the activity of mentioned enzymes. The dilutions of guaiacol were prepared in decimal potentions from 1 to 20 in 95% ethanol. Every two days 0.05 cm^3 of ever solutions was added to separate fungal cultures which were grown in liquid medium. The 14^{th} day of growth, the activities of three enzymes were measured in the mycelium and in the medium. The effect of low-doses of guaiacol on the enzymes activity was distinctly visible. As it was shown for *Pleurotus* culture, the 1^{st} , 8^{th} and 15^{th} potentions are profitable for maximum activity of laccase, 8^{th} and 12^{th} activated peroxidase, and 8^{th}, 10^{th} and 12^{th} were the best for catalase activation. So for all enzymes the 8^{th} potency (10^{-8} mg/cm^3) of guaiacol was the best inductor. As the conclusion we can say that the low-dose of guaiacol could be used as the natural aromatic effector, which increase the activity of enzymes connected with the ligninolytic processes.

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9. Ultra-weak Secondary Radiation Chemiluminescence of Humic Acid Induced by Gamma-irradiation

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Dried humic acid and its aqueous solution in were irradiated with doses (1-10 kGy) of g-irradiation. A secondary ultraweak long wavelength radiation (340-650 nm) from the irradiated solutions, but not from dried humic acid was recorded as a long-living chemiluminescence. The spectrophotometric measurements of the irradiated solutions indicated on the degradation of humic acids macromolecules. The effect of chemiluminescence enhancer - luminol on the intensity of chemiluminescence proved the participation of reactive oxygen species and a free radical mechanism of the degradation processes. The effect-dose functions have clearly a non-linear shape suggesting complex mechanisms underlying chemiluminescence and oxidative degradation reactions. The significance of the observed phenomena for their possible positive ecological consequences as well as for the radiation hormesis is shortly discussed.

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10. Linear and Non-linear Effects of Lead on Behavior of *Drosophila melanogaster*

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Environmental lead is a serious neurotoxin, causing hyperactivity and cognitive deficits in children. Our study of such would be facilitated by having a model system we could manipulate easily and quickly. We find *Drosophila melanogaster* ideal as such, and we have been studying effects of lead on locomotor activity, courtship and fecundity. We raised Canton S flies from egg to mature adult on medium made with lead (2 ppm to 50 ppm lead acetate solution), or with distilled water. Locomotor activity was measured with an Aopen field@ test: individual flies were transferred to a grid-labeled petri dish and the number of lines crossed in 30 s was counted. Copulation rates were measured with a group mating test: five females and five males were transferred into an empty vial and the number of pairs *in copula* was recorded at 5 minute intervals for 20 minutes. Fecundity was defined as all adult offspring from eggs produced by one female in twelve days post-mating. Some behaviors (locomotor activity) varied linearly with lead dose; others (copulation rates, fecundity) did not. Locomotor activity rates in males were consistently higher than those in females, but both decreased significantly with increasing lead dose. Copulation rates increased at low lead doses (2 & 8 ppm) but decreased at higher ones (10 to 50 ppm -- the 50 ppm group were not significantly different from the controls). Fecundity was significantly higher in the low-lead (2 ppm) group and returned to control levels in the high (20 ppm). The non-linear low-lead effects on copulation rate and fecundity are examples of *hormesis*, a commonly observed stimulatory effect of low doses of toxins. We hope from further studies with *Drosophila* to understand better how hormetic effects of pollutants might affect fitness of organisms and ecosystems. Supported by a Whitehall Foundation grant to HVBH.

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11. Extracelluar and Intracellular Replication of Bacteria in Presence of Cytokines or Lipopolysaccharide(LPS): Linear and Non-Linear Responses

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Innate host immune response manifested by elaboration of key inflammatory molecules such as IL-1β, IL-6 and TNFα help in eradication of invading pathogens. However, certain disease conditions such as acute respiratory distress syndrome (ARDS) were shown to have persistent elevated levels of proinflammatory cytokines mentioned above and increased incidence of bacterial infections. We therefore investigated the relationship between the presence of proinflammatory cytokines and bacterial growth both in the extra and intracellular milieu. We designed in vitro studies to mimic the course of events with respect to microbial adherence, internalizations, intracellular replication and extra cellular replication in relation to concentrations of the key inflammatory molecules mentioned above or lipopolysaccharide(LPS) which is known to induce the expression of these proinflammatory molecules. We used fresh clinical isolates of *S.aureus*, *Ps.aeruginosa* and *Acinetobactor* sp (frequent isolates from the lung or blood of ARDS patients). A linear relationship was observed in terms of the extracellular replication of bacteria and the cytokine concentrations. The adherence (assessed by a modified ELISA), intracellular survival and replication (as assessed by the number of viable bacteria after each experiment within and outside the cell) showed a U-shaped curve, indicating that the responses were not linear: The bacteria tested survived when the cells were exposed to low concentrations of cytokines or LPS, they also survived and replicated efficiently when the cells were exposed to “supraoptimal” concentrations of the tested cytokines or LPS. However, the microbes were effectively killed at “optimal” concentrations of the priming cytokines or LPS. We believe that biological responses are not always necessarily linear with respect to the intervening agent(s). These observations are likely to have implications in designing and evaluating therapeutic regimens or biological modifiers to counteract disease processes.

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12. A Mathematical Model for Curve-Fitting Allelochemical Dose-Responses

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Bioassay techniques are often used to study the effects of allelochemicals on plant processes and it is generally observed that the processes are stimulated at low allelochemical concentrations and inhibited as the concentrations increase. The dose-response relationships are usually inverted U-shaped (hormesis) in the science of allelopathy. A simple empirical model is presented to analyse this type of response. The stimulation-inhibition properties of responses can be described by the parameters in the model. The indices, *p%* reductions, is calculated to assess the allelochemical effects. The model is compared with experimental data for the response of lettuce seedling growth to Centaurepensin, the olfactory response of weevil larvae to a-terpineol, and the response of annual ryegrass *(Lolium multifio- rum* Lam.), creeping red fescue *(Festuca robra* 1., cv. Ensylva), Kentucky bluegrass *(Poa pratensis* 1., cv. Kenblue), perennial ryegrass *(L. perenne* 1., cv. Manhattan), and Rebel tall fescue *(F. arundinacea* Schreb) seedling growth to leachates of Rebel and Kentucky 31 tall fescue. The results show that the model gives a good description to these sets of data and can be used to fit a wide range of dose responses. Assessments of the effects of leachate of Rebel and Kentucky 31 tall fescue clearly differentiate the properties of the allelopathic sources and the relative sensitivities of indicators such the length of root and leaf.

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13. Nectophotometer: A New Instrument for Observing Hormesis

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An instrument and method developed to monitor toxicity to motile aquatic organisms has proven useful for demonstrating hormesis. The Nectophotometer monitors the movement of organisms exposed to toxicants and allows recorded changes in movement over short time intervals to be used to predict mortality after prolonged exposure. In all tests a toxicant that causes mortality following prolonged exposure (72 hours) is found to stimulate test organisms during short-term exposure (2.5 hours and less). Correlations between activity levels during initial exposure to each concentration of a toxicant and subsequent mortality in that concentration show the hormetic effect contributes to the prediction of mortality. Since toxicity studies consider the duration of exposure in addition to dose and response our data demonstrates an important dimension of the hormesis phenomenon - stimulation at low duration exposure and inhibition at high duration exposure. The Nectophotometer will also be useful for evaluating the effects of exposure frequency on hormesis.

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14. Adaptive Response in Two Populations of Uranium Miners Exposed to Low and High Doses of Radiation

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We compared the capacity of adaptive response to radiation, from peripheral blood lymphocytes of workers, low and highly exposed.

The adaptive response for radiation-induced chromosomal aberrations was studied in two populations of miners, working in Portuguese uranium mines. They had received during a period of fifteen years of work, less than 10 cGy and more than 30 cGy respectively. The individuals of both populations were matched by age, sex, and lifestyle habits (smoking, drinking, etc…). The *in vitro* induction of the adaptive response, on the peripheral blood lymphocytes, was made with a dose of 5 cGy of gamma rays on G1 phase cells, and with a challenge dose of 300 cGy on G2 phase cells.

The chromosomal aberration analyses showed that there was induction of adaptive responses on all miners less exposed to radiation (<=10 cGy), and that all but one of those who had received higher doses (>=30 cGy), didn't show capacity for induction of adaptive responses. With the caution of the small number of cases on this study (20), these data point in the sense that the lower doses demonstrate a higher capacity for expression of adaptive response than larger doses.

The adaptive response radiation seen at low doses may increase the capacity of DNA repair and may reduce the risks of cells to be transformed in cancer.

So, our data does not support the linear no-threshold hypothesis on radiation protection that assumes that every dose, no matter how low, produces some risk.

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15. Bimodal Type of Regulation of Protein Kinase C Activity by Antioxidants Down to Ultra-Low Doses

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Antioxidants control lipid peroxidation in general and can act as effectors of membrane-bound enzyme in particular. Protein kinase C (PKC) - peroxilipid-dependent enzyme is one of the most important one involved in regulation of a number of cell functions. Purpose of this study was: 1. can antioxidants regulate PKC activity as affectors; 2. what concentration range of antioxidant effect on PKC activity; 3. is it possible to model the effect obtained in experiment. The action of two different antioxidants - natural lipid-soluble α-tocopherol (α-TL) and synthetic water-soluble phenozan potassium salt (PhK) in a wide range of concentration (10^{-3} M - 10^{-20} M) on PKC activity has been studied. It was shown that α-TL inhibits (60-80%) purified enzyme isolated from rabbit hearts (α-TL) and PhK activates (in 4-5 times) PKC activity in normal and tumor cell cultures: rat vascular smooth muscle cells (VSMC) and human osteosarcoma (Saos-2); it was demonstrated that PhK is superactivator of PKC activity. It was found that irrespective of the "sign" of antioxidant action (inhibition or activation) the "dose-effect" curves are of bimodal type with two maxima at 10^{-4} – 10^{-6} M and ultra-low doses of antioxidants 10^{-14}-10^{-18} M , between them – so-called "zone of silence", in which the effect of antioxidants was not observed. In the experiments using α-TL the dependences of PKC activity upon substrate (histone H1) concentration have been shown to be a "bell" type. The kinetic parameters of PKC inhibition by α-TL were estimated using formal kinetic schemes of enzyme reaction, conjugated graphs method and others. It was found that α-TL is non-competitive inhibitor of PKC activity and ultra-low dose changes the main kinetic constant and parameters. A scheme of allosteric regulation of PKC activity by α-TL is proposed. Major conclusion is antioxidants can regulate PKC activity not only at high (or physiological) concentration, but at ultra low doses as well.

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16. Adaption to Radiation: Reductions in Risk after Low Doses *in vivo*

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The “Linear No Threshold” hypothesis, used in all radiation protection practices, assumes that all doses, no matter how low, increase the risk of cancer. We have used animal experiments to test this hypothesis *in vivo. A* single, low dose rate, whole body dose (100 mGy) of low LET radiation given to genetically normal, adult mice did not alter frequency but did increase the latency for acute myeloid leukemia, initiated by a subsequent large dose. This indicates that a low dose reduced the rate at which initiated cells became genomically unstable, and consequently reduced risk. This reduction in the progress of genomic instability also occurred in radiation sensitive, cancer prone *Trp53* heterozygous mice, where a single 10 mGy dose again had no effect on spontaneous cancer frequency, but significantly increased latency for spontaneous osteosarcomas, lymphomas and hemangiosarcomas. The protective effect of this adaptive response against spontaneous cancer lasted for the entire lifespan of all the animals that developed these tumors, effectively restoring a portion of the mean loss of life attributed to *Trp53* heterozygosity in the absence of radiation exposure. Increasing the dose to 100 mGy increased risk (decreased latency) for some tumors but increased latency for other tumors, indicating a protective threshold was being approached. In fetal mice, low doses (30 cGy) prior to a large 4 Gy exposure also protected against teratogenic effects resulting from both *Trp53* dependent and independent apoptotic processes, but the protective effects varied with both *Trp53* status and gestational time. In genetically normal mice, 100 mGy given to male mice the day before a 1 Gy exposure reduced heritable mutations in the offspring of the irradiated mice. Overall, the results demonstrate that the assumption of a linear relationship between dose and risk *in vivo* is not warranted, and that low doses actually reduce risk.

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17. The History of Chemical Hormesis and Potential Implications for Modern Risk Assessment and Epidemiology

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Low dose stimulation coupled with high dose toxicity was recognized by the 1880's. Work by Hugo Schulz and Rudolph Arndt led to the "Arndt-Schulz Law," expanded as "Hueppe's Rule" in the 1890's. By 1905 the first beta-curve was published, and this concept developed on several different fronts. The modern term "hormesis" was coined by Southam and Erhlich in 1943 to describe low dose stimulation, but more recently has been associated with an over-compensation response to a disruption in homeostasis.

Despite the long history of hormesis-related experimental research, few systematic efforts to describe its history are available. This presentation reconstructs the history of such research and evaluates how advances in related scientific fields affected the course of hormesis-related research, and the nature of the bioassay in the modern risk assessment framework. More recent research suggests that hormetic effects are commonly observed by established scientists, and are often published in peer-reviewed journals. Mounting evidence suggests that hormesis is generalizable across animal models and biological classes, and may be highly relevant both conceptually and methodologically to epidemiology and risk assessment today.

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18. The Development of an Reproducible Toxicological Bioassay to Elucidate Hormesis

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The goal of the research was to evaluate hormetic responses within an established insect model, and develop a reproducible bioassay to evaluate the physiological effects of low-level environmental stress. This study was designed to determine if *Phormia regina* exposed to cadmium chloride during larval development display a developmental advantage, based on mean survival data of both pupae and adults, when compared to concurrent vehicle controls. Initially, two methodological approaches were considered in assessing which environmental stressor may have an hormetic effect on developmental parameters. One was to expose adults to a gamma ray source, while the second was to administer adults a selected metal chloride via a single or multiple injection to the abdomen. While the gamma ray source (Cs-137) exposure studies displayed potential for follow up research based on preliminary investigation, they are not further discussed due the relative success of an alternative larval fly model. The initial intent to expose adult *Phormia* to metals via subcutaneous abdomen injections proved problematic with respect to the amount of time required, and the additional stress imposed on the organism. Concurrent controls treated with a sham injection experienced high mortality relative to non-treated controls. Pilot studies conducted on the effects of carbon dioxide and ice anesthetic treatments (required for abdomen injections) revealed a significant difference between ice/CO_2 treated flies and untreated controls. These complications facilitated a re-examination of the exposure procedure and brought about a new study design incorporating a larval diet exposure. Hormetic effects are typically subtle (sometimes +/- 20% of the control) and a decision was made to minimize any physiological insult so that any possible hormetic effects from the administration of a low dose metal would not be confounded by administration stress. The larval diet exposure was found to be favorable due to the non-invasive, continuous administration of metal, the absence of any anesthetic agents (such as ice and carbon dioxide) and the relative ease of experimentation. The blowfly model system employed in this study has revealed a hormetic dose response across various developmental stages and two physiological stressor agents (cadmium and gamma radiation). This model has also revealed an unexpected result of stage specific mortality observed in the transition of larvae to pupae, as well as in the transition of pupae to adult. These preliminary data suggest that the blowfly larvae model may provide an ideal bioassay for elucidating possible hormetic effects, and developmental susceptibility to environmental contaminants.

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19. Continuous Low Dose-Rate Gamma-Irradiation Induces Non-Linear Changes of DNA-Protein Cross-Links in Lymphocytes of Mice

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DNA-protein cross-links (DPC) are an important type of DNA damage induced in cells by some chemical and physical agents. The most studies of DPC formation were performed at the action of high doses of cross-linking agents. The data on the formation and the role of DNA-protein cross-links at exposure of organism to the genotoxicants at low doses are scarce and controversy. Extensive debates still take place about the shape of dose-response relationships in repair-proficient normal cell in region of very low doses. In this report we demonstrate the results of DPC induction study in spleen lymphocytes of CBA line mice under continuous (in course up 1 year) exposure to very low dose-rate gamma-radiation (0.72 mGy/day and 1.7 mGy/day). Our investigations showed that: 1) the dependence of the DPC level in spleen lymphocytes from total dose (exposure time) is non-linear; 2) there is no dependence of the effect from dose rate; 3) changes in the DPC levels are associated with these of spleen lymphocytes' total number. It is known that chronic exposure to ionizing radiation at low doses leads to non-linear changes of some metabolic parameters of cells. Our studies continued over a long period of time strongly support the idea that DPC participate in overall response of the exposed cells to low doses of ionizing radiation. It is not yet known whether DPC cause alteration in gene expression and present the factor of risk.

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20. The Effect of Antioxydants in Ultra Low Doses on Lipid Peroxide Oxidation in Biological Membranes

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The effect of natural lipid-soluble antioxidant, α-tocopherol (α-TL), and the synthetic water – soluble antioxidant, phenosan potassium salt (Ph-K), in a broad range of concentrations down to ultra low doses (10^{-20} – 10^{-4} M) on the models of spontaneous and initiated lipid peroxide oxidation (LPO) in endoplasmic reticulum membranes from mice liver have been studied. LPO modification was registered both by the increase in the content of primary (hydroperoxides - HP) and secondary (malonic dialdehyde, MDA) oxidation products and by the consumption of the unsaturation substrate (total double bond –DB – content). α-TL and Ph-K were shown : a) to inhibit spontaneous and initiated LPO estimated on the basis of three criteria.; inhibition percentage is changed from 30 to 90% in dependence from concentration; b)the dependence dose – effect has poli - or bimodal form which is characteristic feature for substances effecting in ultra-low doses. The data are confirmed by the character of the oxidation velocity dependence on α-TL and Ph-K concentrations. There is a significant difference in the case of using MDA as an LPO characteristic for experiments with Ph-K : the change of the sign of effect takes place – the inhibiting effect at the range of concentrations (10^{-4}-10^{-10} M) transforms into promoting action at (10^{-12}-10^{-15}M). Such form of the curves is typical for the different agents with action of low intensity, but it is observed more seldom than bi- or polimodal type of curves. It is also typical for the both curves the existence of the " silence zone" between the maxima in the range of low and ultralow antioxidant concentrations, the silence zone covers 1-4 orders of antioxidant concentrations. Thus, we can conclude, that both as natural as synthetic antioxidants are the typical agents showing effect in ultra-low doses.

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21. Nonlinear Dose-Response Behavior and Multiple Solutions for a Model of the Menstrual Cycle

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A mathematical model of the menstrual cycle has been developed based on the premise that the underlying dose-response relationships for individual components are continuous and monotonic, although nonlinear. The purpose of this model is to aid in the understanding of menstrual cycle regulation and to predict the effect of exposure to exogenous hormonal compounds. Initially, two components were developed. The first component represents the pituitary, secreting luteinizing hormone, and follicle-stimulating hormone. The second component represents the ovaries, secreting estradiol, progesterone, and inhibin. When the two components were combined, the model produced a solution that closely approximates the normal menstrual cycle. However a second, unanticipated solution that is clearly abnormal was also found, with hormone levels similar to those in women with polycystic ovarian syndrome (PCOS). Simulations of exogenous perturbation of the two solutions show that the system returns to the original solution after small perturbations. But sufficiently large perturbations can cause the model to permanently shift from the normal to the abnormal cycle or vice-versa. For example, a 5-day infusion that increases the circulating level of progesterone by 80 nmol/L during the early luteal phase of the abnormal cycle causes the model to jump to the normal cycle; if the infusion is only 30 nmol/L, then the solution returns to the abnormal cycle afterward. On the other hand, if 30 nmol/L are infused during the luteal phases of two consecutive cycles, the model will subsequently goes to the normal cycle. Thus the model exhibits biphasic behavior, or a switch, although each component process has continuous, monotonic dose-response behavior. With further development, the model is expected to be useful in estimating health risks from environmental exposure to endocrine-active compounds and in biomedical applications such as designing birth control regimens and therapeutic interventions for diseases such as PCOS.

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22. Extremely Low Frequency Electormegnetic Field (ELF-EMF)-Sress Induced DNA Damage in Human Peripheral Blood Leukocytes Evaluated by Comet Assay

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A wide variety of environmental stimuli are known to induce the expression of stress response genes. One of the expressions of stress response is DNA damage. To address the question if extremely low frequency electromagnetic fields (ELF-EMFs) induce a stress-induced DNA damage, the present study was carried out. *Invitro* effect of ELF-EMF from 0.2 mT to 1mT (5 flux densities) at 50 Hz on DNA integrity in human peripheral blood leukocytes was studied. The experimental setup to produce the ELF-EMF consisted of an assembly of a dimmerstat, transformer, ammeter and air-cored coils to provide the seat for the blood samples. The samples were placed inside shielded *mu*-metal box kept in an incubator at 37^{o} C. Comet assay was performed on heparinized peripheral blood leukocytes of six adult males exposed to the varying flux densities mentioned above. Zero flux density served as control. 100 cells per treatment were scored for comet tail-length, which is an estimate of DNA damage. With increase in magnetic field (MF) flux density there was a stepwise increase in DNA damage. The increase in DNA damage seen in our experiment is probably because of stress response due to ELF-EMFs.

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23. Non-Linear Dose-Response of Alcohol Drinking to Mortality in a Large Population

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We studied subsequent mortality in relation to alcohol habits reported by 128,934 persons at health examinations in California from January 1978 through December 1985. Persons classified themselves with respect to race, marital status, education, cigarette smoking, and other traits, and also with alcohol intake as lifelong abstainers, ex-drinkers, and drinkers of the following groups: <1 drink/month, 1 dr/mon to <1 dr/day, 1-2, 3-5, and ≥6 dr/day. Drinkers received separate questions about the number of days per week they drank wine, liquor, or beer. The traits of persons preferring wine indicated, in general, a probable more favorable risk of illness. Over a mean follow-up of 8.0 years, there were 4501 deaths (1685 cardiovascular [CV] and 2638 non-CV). Multivariate Cox proportional hazards model analysis with lifelong abstainers as reference showed a J-shaped overall alcohol-mortality relation, with important sex and age differences for heavier drinking (women and younger persons at higher risk). Apparent benefit of lighter drinking was most evident at age≥60. Most of the lower mortality risk of lighter drinkers was due to lower CV risk, especially lower coronary heart disease risk. The increased risk of heavier drinkers was due to a variety of CV and non-CV causes, especially cirrhosis, unnatural death, and certain cancers. Analysis of the role of beverage choice showed slightly lower total mortality risk (RR=0.9, NS) for preferrers of beer or wine, and significantly lower risk for CV death (RR=0.7; p<0.01) for preferrers of wine, compared with liquor preferrers. Conclusions: Women and younger persons appear more susceptible to the increased mortality risk of heavy drinking. The reduced cardiovascular risk of lighter drinkers is more pronounced in older persons.

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24. Season of Birth and Human Longevity in a Large Population

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Recent research suggests that adult age at death is associated with the season of birth. Data for Europe and Australia are consistent and show that people born in winter in average live longer then people born in summer. We analyzed 579,141 people who died in California in 1966-1999 at age 15 or greater of non-accidental causes. Our study results show that people born in February through April on the average had slightly (but significantly) longer life span than those born in June through November. A difference in life span between people born in February and November averages up to 1.25 year. A 1-year cycle was found by cosinor analysis in longevity variations by month-of-birth for the whole cohort (men: $P<0.0001$, women: $P=0.002$). A multivariate Cox proportional hazards model analysis also showed statistically significant month-of-birth longevity dependence when adjusted by sex, ethnicity, and place of birth. The observed seasonal effects were similar for men and women, various causes of death, and were different for various age groups, skin colors, and geographical places of birth. Observed difference for skin color groups may be explained by difference in place of birth (most of Asians were born in Asia, most of Blacks born in other states). “Latitude” hypothesis is not supported by observed difference in seasonal effects for northern and southern areas. Probably other seasonal climatic, weather, and nutritional factors should be considered. Observed geographical effects require further investigation. Observed seasonal effects support the hypothesis that the environmental conditions during the first 3 months of human development affect the later health and longevity. Possible seasonally related environmental exposures, such as seasonal variations in micronutrient and vitamin intake or infectious diseases, might affect human survival in later life.

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25. Gamma Variate (Indicator Dilution Curve): Analogy to Describing Selected Hormesis Effects

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The concept of hormesis requires initial efforts at quantification, in an attempt to i) describe the events with a minimum of variables, and ii) visualize the magnitude of separate factors. There are at least 2 phenomena which have to be taken into account. The first is a stimulatory effect at low doses of the agent (such as chemical of radiation). The second effect is a decreasing action at higher doses or increasing time. In terms of mathematical representation, this bears a resemblance to the indicator dilution curve (gamma variate).

$$\text{Effect} = A \cdot t^{B} \cdot e^{-Kt}$$

That is, there is an initial upward sweep of the curve, followed by decreasing values. If the scale employed is time, the down slope can represent a washing out of the stimulatory agent or an increased catabolic pathway. If the scale represent biological effect, it can follow from the concentration of the agent (with or without a time lag entered into the equation). It is apparent that both stimulatory and "fall off" effects must be considered when examining the hormesis phenomenon. Further examination will likely yield more descriptive equations.

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