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The dose response principle from philosophy to modern toxicology: The impact of ancient philosophy and medicine in modern toxicology science

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ABSTRACT

Since ancient times the concept of dose response, from a toxicological perspective, has been a matter of concern. Already by the 8th century BC and over the years, many enlightened people have attempted to interpret this phenomenon, observing and coming across its results and practical implementation through exposure to chemical substances, either from natural or synthetic sources. Nowadays, the environmental exposure of human populations to chemicals in terms of quantity and quality might differ. Nevertheless, dose response still remains an issue joining hands with scientific and technological progress. The aim of the present review is not only to briefly recount the history of the dose response concept, from ancient time theories to novel approaches, but also to draw the outline of challenges and requirements toxicology science needs to fulfill.

1. Introduction

The notion of “dose-response” has been described since antiquity. Indeed, many Greek and Latin adages attest this concept. Hesiod (8th century BC) (Fig. 1), in *Harmonia*, refers to the notion of ‘moderation and harmony’. An inscription in the Apollo Temple in Delphi (600 BCE) states ‘meden agan’ (μηδὲν ἄγαν), an equivalent for ‘nothing too much’ (Chilon of Sparta, 6th century BC, Fig. 2). Cleobulus of Lindos (625-555 BCE) (Fig. 1) stated the quote ‘metron ariston’ (μέτρον ἄριστον), namely the optimal is the right measure. In Roman times, the playwright Terentius (190-159 BCE) said that ‘ne quid nimis’, meaning that nothing that is too much, is the best. Horace (65-8 BCE) in Odes 2.10 (c. 13 BCE) postulates the proverb ‘aurea mediocritas’, which can be translated as the golden middle. Another Latin proverb states that ‘in medio virtus’, which equates to the notion that the virtue is in the middle, and ‘minima maxima sunt’, meaning that the minimal is the maximal.

Hippocrates (460-377 BCE) (Fig. 2), a Greek physician, is often referred to as the "Father of Western Medicine". We know today that the response to a particular dose of a particular substance can greatly vary from one individual to another. The possible reasons include genetic susceptibility, interactions between different drugs, or even receptor differences that lead to vulnerability [1]. But what we know today about personalized response to a substance was already suggested by Hippocrates thousands of years ago [2]. In the Hippocratic Corpus (*Corpus Hippocraticum*), which is a collection of seventy medical works collected in Alexandria, Hippocrates described the individuality of disease which leads to the necessity of giving "different [drugs] to different patients, for the sweet ones do not benefit everyone, nor do the astringent ones, nor are all the patients able to drink the same things." When choosing the drugs to be prescribed, Hippocrates evaluated different factors such as a person’s “constitution”. We know today that this is all about DNA and following the completion of the Human Genome Project, more and more

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Fig. 1. (*left*) Hesiod, Greek poet (*Theogony & Works and Days*), (*middle*): Mithridates VI, king of Pontus and a pioneer of toxicology, (*right*): Cleobulus of Lindos, poet and one of the Seven Sages of Greece.

personalized, gene-based, therapeutic approaches are being developed. Hippocrates also highlighted the influence of the environment on health, setting the fundamentals of what we know today as epigenetics. Indeed, current scientific evidence has shown that environmental exposure can up- or down-regulate particular genes [3] and can therefore influence the response to a particular dose.

In his treatise Air, Water and Places, in 400 BCE, Hippocrates stated that the appearance of disease in human populations is influenced by the quality of air, water and food, the topography of the land, and general living habits [4]. His contribution also regards the formulation of the first thoughts on toxic properties of metals during professional exposure in lead miners of ancient times [5].

The ancient Olympic Games were one of the first applications of pharmaceutical toxicology using natural performance enhancing drugs, where dose response plays a crucial role, both for the enhancement of performance and the minimization of the adverse effects. Hallucinogenic mushrooms and naturally derived stimulants were widely used to overcome fatigue or injuries. Even in 700 BCE there was an awareness that heightened testosterone would increase performance. With no syringes or hormones in injectable liquid form, it was left to the athletes to gorge on animal hearts and sheep testicles in search of potency. Athletes could also eat a delicacy we know today as Rocky Mountain oysters. And in that way, they would boost their levels of testosterone. Aretaeus of Cappadocia, a 1 st century ancient Greek physician, once opined on the purportedly salubrious effects of such ingestion: “For it is the semen, when possessed of vitality, which makes us to be men, hot, well braced in limbs, well voiced, spirited, strong to think and act. … But if any man be continent in the emission of semen, he is bold, daring, and strong as wild beasts as is proved from such of the athlete as are continent. … Vital semen, then, contributes to health, strength, courage, and generation.”

Athletes competing at the Games did not need to look far for assistance. Cooks and doctors were often willing to offer their expertise when it came to supplements and prescribe the exact dosage scheme, as many of the potions could have deleterious effects, too. There was an array of fungi, herbal medications and potions – often hallucinogenic or poisonous – that were taken in an attempt to gain the upper hand. In the Third Century BC, athletes tried to boost their performance using mushrooms. Philostratus (ca. 170-247 AD) reported that doctors were significantly helpful in athletes’ preparation for the Games and cooks prepared bread with analgesic properties containing opium from poppies. In the First Century AD, it was also reported that the Greek runners were drinking an herbal beverage to increase their strength and to be capable of competing in the long duration events, namely extracts of horsetail plants for increased muscle mass and strength. Galen (130-201 AD), another prominent ancient Greek physician, is said to have prescribed “the rear hooves of an Abyssinian ass, ground up, boiled in oil, and flavored with rose hips and rose petals” for a performance-enhancing tonic. Athletes were also known to drink “magic” potions and eat exotic meats in the hopes of gaining an athletic edge on their competition. Dried figs, wine potions, herbal medications, strychnine and hallucinogens were also used.

Mithridates VI Eupator (132-63 BCE) (Fig. 2) was the King of Pontus and of the region of Northern Anatolia. He was one of the most potent opponents against Roman expansion. Three wars have been recorded against the Romans, the 1st Mithridatic War in 88-84 BCE, the 2nd in 83-81 BCE and the 3rd in 75-63 BCE.

Mithridates has been described as a pioneer of clinical toxicology. In an attempt to prevent political enemies from assassinating him through poisoning (Plutarch, *Parallel Lives*, Life of Pompey XXXII, 37), he mixed the blood of Pontic ducks, whose flesh was toxic from ingestion of plants poisonous to humans, with other substances reputed to expel poison (Aulus Gellius, *Attic Nights* 17.16). Mithridates is reported to have acquired immunity to deadly doses of arsenic by ingesting miniscule amounts of arsenic over many years (Dio Cassius, *Roman History* 37.13). It seemed that dividing a dose into aliquots enabled the action of redeeming mechanisms, as it offers adequate time for repair before the next dose is administered. While an entire dose might be hazardous if ingested at once, its fractioning could render the substance less toxic. He titrated himself to various poisons by taking small doses every day, the mithridatium (Pliny the Elder, Natural History 25.3). Mithridates, through experimentations, developed a daily regimen of taking poison along with ‘remedies’ (Appian, *Mithridatic Wars* 12.16). He was known to demonstrate his immunity to poison conspiracy attempts, at banquets, by inviting his guests to apply hazardous mixtures in his food and drink [6]. Later, when he understood that he was falling into the hands of his enemies, he attempted to commit suicide, but due to his tolerance, the amount of poison he swallowed was not effective. Consequently, he took to the sword.

His ‘theriac’ recipe was said to contain more than 50 ingredients, consisting of poison counteracting drugs. Theriac (< theria = wild beasts) was initially administered as an antidote for curative purposes [7]. The first formulation was developed by Asclepios (Pliny the Elder, Galen of Pergamum). Crateuas, Mithridates’ personal physician, created an antidote reported to protect against venoms of scorpions, vipers and sea-slugs. Following Mithridates’ death in Pompey, the formulae of Mithridates were translated into Latin. The original formula of mithridatum has not survived [8]. However, Andromachus concoction, Galene Theriaca, an improved version of Mithridates’ elixir, has survived [9].

Mithridates was the author of a book on roots and plants, which at the time was the most important source of toxins. His interest in clinical toxicology was also demonstrated by experiments he conducted on

Fig. 2. (*left*) Chilon of Sparta, politician and philosopher, and one of the Seven Sages, (*right*) Hippocrates, the Greek physician called 'The Father of Medicine'.

prisoners, in order to test poisons and antidotes. Mithridates came across a paradox, still studied today: lethal toxic substances can be beneficial when ingested in microscopic doses. It is reported that Mithridates acquired resistance to poisoning possibly by enzymatic activation or metabolic functional changes (mithridatism), by taking progressively increasing sublethal doses. For instance, tiny amounts of arsenic over time lead to hepatic production of enzymes, for the neutralization of a normally lethal dose. Mithridatism is not effective against all types of poison. Depending on the toxin, this practice might lead to lethal accumulation of poison in the body. He, however, should be given some credit for developing the ideas of tolerance, hormesis and immunity.

Tolerance is defined as the observed reduced reaction to an administered substance subsequently to its repeated use. Thus, a higher dose is required to achieve the same effect. General tolerance means that all observable effects are eliminated to the same extent, while specific tolerance is specific for a particular effect. Its (dis)appearance may occur at different rates.

Mithridates VI Eupator could be also deemed as the forerunner of ASIT (allergen specific immunotherapy), as it appears to represent a very broad view of the concept of *mithridatism*. This belief presumes that repeated oral consumption of (small) amounts of antigen, regarded as a poison or toxin, induces tolerance or resistance to the antigen itself by predominantly immune mechanisms [10]. Sensitization leads to the establishment of IgE^+ memory B cells and allergen-specific memory T cells. Subsequent repeated allergen contact will boost IgE^+ memory B cells that receive T-cell help to produce increased levels of allergen-specific IgE antibodies [11]. Allergen specific immunotherapy and high-dose encounters with allergens induce T*reg* cells, which leads to peripheral tolerance. The effector cells of allergic inflammation are regulated by regulatory and suppressive functions of T*reg* cells, which suppress Th2 cells and their cytokine production, both for the differentiation, survival, and activity of mast cells, basophils, eosinophils, and mucus producing cells and for tissue homing of Th2 cells [12].

Paracelsus (1493–1541), whose full birth name is Philippus Aureolus Theophrastus Bombastus von Hohenheim, was the first to express the opinion that the dose was the most important factor to define the toxicity of a substance. He also suggested that exposure occurring at early developmental stages is crucial and the importance of genetic predisposition to toxic outcomes. Paracelsus, by his insightful observations, paved the way for the implementation of the NOAEL in pharmacology and toxicology.

The dose response is probably the most pivotal notion in toxicology, and its basis lies on observations and data retrieved from research, both in animals and humans. It associates exposure with a versatile outcome of results. It has been reported that the concept of the dose response was initially put forward by the French physiologist Claude Bernard (1813–1878). Other researchers have extended this concept to include the excretion of various metabolic products. The threshold perspective was placed within a broader context by the pharmacologist and physiologist Arthur Robertson Cushny (1866–1926).

According to Erich Harnack (1852–1926), a German physician, the minimum level of observable action that a drug could induce, can be called the *minimal effective dose*. Supplementary to that, Paul Ehrlich (1854–1915) postulated the term *minimum lethal dose*. Both Ehrlich and Harnack attempted to state descriptions regarding tolerance levels. Shackell, during the 1920s, reported that the dose response curve typically presents a sigmoid shape [13]. The point on the curve below where toxicity first manifests itself is the threshold dose level (Fig. 3).

A toxic dose is defined as the quantity of a substance that will produce a harmful or untoward effect. Included in the concept of a toxic dose is the amount of a chemical administered and entering the body and depending upon the concentration and the properties of the toxicant, the timing and frequency of exposure, the length of exposure and the exposure pathway. Multiple types of doses occur, including the exposure dose, the absorbed dose, the administered dose and the biological effective dose. The term response refers to the degree of reaction, related to the dose and the organism.

For prediction of toxicity of a substance, the shape and the slope of the curve are important additional information. The slope indicates the percent of population responding per unit change in dose. In general, the dose response pattern enables the determination of the existence of effect in a given dose, the threshold of effect, and the rate of progress of toxic effects, namely the slope.

Exposure can occur in high or low levels; similarly, the response outcome can be acutely manifested, or appear after a period of time, bringing about tumorigenesis and degenerative diseases. It is thus evident that different types of responses are related to various exposures. Exposure pathways include respiration (gases and particulate matter), oral (usually drugs, environmental pollutants) or via the skin (pesticides, environmental pollutants). Parameters that affect the toxicity of a substance are, apart from its physical chemical traits, the dosage and the time of exposure, the route of exposure, gender [14], age and health of the individual, as well as absorption, metabolism, distribution and

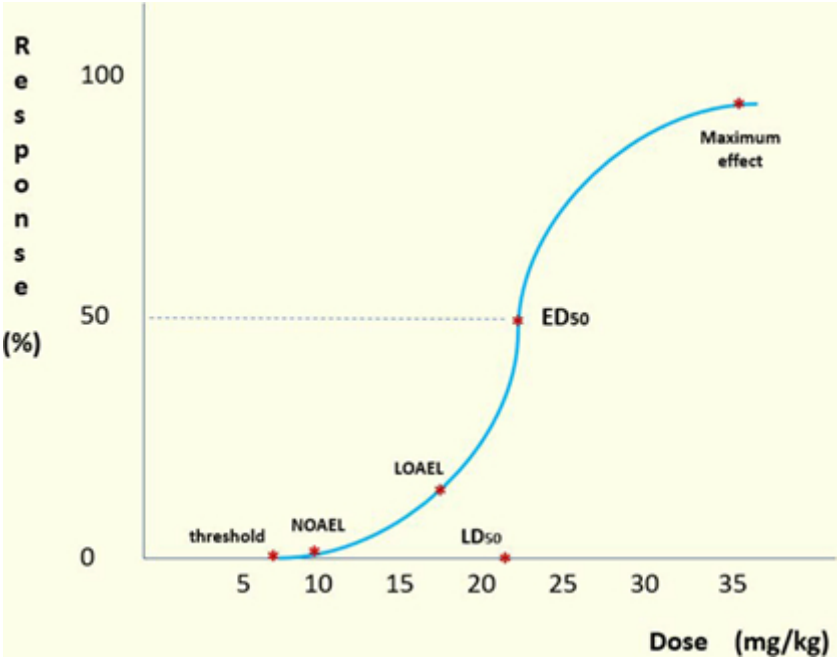

Fig. 3. Dose response sigmoid curve. NOAEL; No-observed-adverse-effect level, LOAEL; lowest-observed-adverse-effect level, ED_{50}; median effective dose, LD_{50}; median lethal dose.

excretion [15–17]. The presence of other chemicals can also affect toxicity as they can eliminate, add or augment toxicity, namely antagonism, additivity and synergism [18].

Toxic properties of chemicals can be described by the quantification of the dose response characteristics. Examples are the point where an environmental factor becomes toxic, or the potency that a stimulus possesses in order to surpass the homeostatic mechanisms and impose cellular damage. Consequently, toxic effects include cellular, biochemical and macromolecular changes, like disturbance of cellular mechanisms (enzymic system lesions, hindrance of protein synthesis), production of reactive oxygen species and DNA damage. The outcomes occurring after exposure to a toxicant among a population are quite consistent, as the majority presents similar responses; there are though individuals that exhibit a different response, depending on the susceptibility of an individual to the particular toxicant. These variations in a population can be depicted in a Gaussian distribution, where one standard deviation represents approximately 68% of the population, while two standard deviations represent 95% of the response. Beyond those percentages, variability of responses increases or decreases depending on the individual [19].

Significance of occupational medicine and also occupational hygiene should not be ignored when considering dose-response phenomena. It was Alice Hamilton, a USA pioneer of occupational medicine, who in the *American Journal of Public Health* in 1914 summarized the evidence for industrial lead poisoning [20]. More importantly, this article was a call to action, directing physicians responsible for workers' health to leave the comforts of their offices to observe the work processes of their patients and to learn the truth about workplace exposures. In this way relationship between dose and exposure was slowly understood among scientists. A particularly important yet at that time confusing term in toxicology was the threshold, meaning the level of exposure at which an effect was first observed. However, the existence of thresholds for certain types of response like carcinogenicity is controversial. In spite of all scientific research during the past century, no single method has been invented to assay total exposure and its clinical effects at the same time. Most often clinical exposure-response relationship was applied for occupational health purposes and surveillance. The third type of exposure-response relationship relates exposure levels to the frequency of the response in a population and can be called epidemiologic relationship. Dose assessment in laboratories is a feasible task to fulfill; however, for the measurement of worksite and environmental exposure in humans, multiple parameters must be taken into account, i.e. chemical concentration, duration and frequency of exposure, thus adding further complications [21].

According to Waddell, after the beginning of the 20th century, advances were reported in the dose response concept, as the scientific community worked to further develop and understand this concept. Pharmacology and toxicology adopted the theory of dose response in the 30's, with the subsequent enactment of appropriate regulations for drugs and chemicals. However, no attempts for investigating influential parameters, like the demarcation of the area inhabited by a population that has been exposed to doses below a certain threshold, had been made [22].

Earlier history of dose-response approaches (1975) can be described as the time of the establishment of the linearized multi-stage model. In the mid-70 s, statistical approaches began to be used, in an attempt for scientists to explain the dose response pattern, especially for chemical carcinogens. It has recently been suggested by Waddell and Rozman that the linear scale used to describe a dose response eliminates the low-doses spectrum so that no precise assessment can be performed [15]. On the other hand, the logarithmic model for dose response more closely relates with the laws of nature. However, the optimal system for dose response scaling seems to be the Rozman scale, which exhibits a combination of both. Thus, it is logarithmic when attributing the dose response and it exhibits linearity for effect and continuity to one molecule, as well as being based on molecule weight. The Rozman scale can also perspicuously depict the threshold regarding tumor formation [23].

1.1. Hormesis

Hormesis, etymologically deriving from the ancient Greek word ὅρμησις, meaning eagerness, urge [ὁρμῶ < ὄρνυμι (set in motion, urge)], is a term used to refer to a biphasic dose response to an agent characterized by a low dose stimulation or beneficial effect and a high dose inhibitory or toxic effect[24]. Typically, low dose exposures incite a beneficial response, while an elevated dose induces toxicity [25]. In the fields of biology and medicine, hormesis is defined as an adaptive response of cells and organisms to a moderate (usually intermittent) stress. Examples include ischemic preconditioning, exercise, dietary

energy restriction and exposures to low doses of certain phytochemicals [26]. Hormesis is not limited to toxicology, pharmacology or medicine but it is a general phenomenon that occurs in most, if not all, scientific fields [23,24].

The term hormesis dates back to 1943, when Southam and Ehrlich found that the extract from a certain tree species augmented fungal metabolism rates in small doses but hindered the metabolism rate at elevated doses [27]. Thus, this stimulation that is observed at low doses that do not exceed a certain threshold is described as 'hormesis'.

The biphasic nature of the dose-response, however, had been reported as early as 1887 by Hugo Schulz (1853–1932), a pharmacologist who came to a related conclusion regarding fungal metabolism. Nine years later, Hueppe stated that toxicants at low doses trigger biological events. Schulz, based on a former hypothesis about the usage of veratrine as a drug that was offering positive results when administered in solution but was ineffective even at very high doses in confronting bacteria, concluded that the nature of dose-response displayed a biphasic pattern, thus suggesting that homeopathic therapy could benefit by this event. The notion of hormesis was thus acquiring its basis, and the phenomenon described above was named the Arndt-Schulz law [27]. Clerk (1885–1941), a reputable professor of pharmacology, rejected the biological dimension of the Arndt-Schulz law. In late 40 s, two researchers, Southam and Ehrlich, were led to similar conclusions to the Arndt-Schulz law, calling their results hormesis. It is interesting to note, that between the 20 s and 40 s, the Arndt-Schulz law had been rejected by eminent researchers, with the assertion that the stimulatory response was only a response to damage/malfunction, rather than a direct result of stimulation. However, in 1929, Branham, based on the Arndt-Schulz law, added the concept of a temporal parameter [28].

Hormesis can be graphically explained, as it can be demonstrated as a U-shaped (or inverted U-shaped) curve, whose limits depend upon the measured endpoint (Fig. 4).

Thus, complementarily to the afore-mentioned, the dose-response relationship can be affected by several factors; the size of the low-dose stimulatory response, the number of doses establishing the reliability to the hormetic curve, the statistical power and the reproducibility of the findings [27].

According to Calabrese & Baldwin, hormesis functions as a

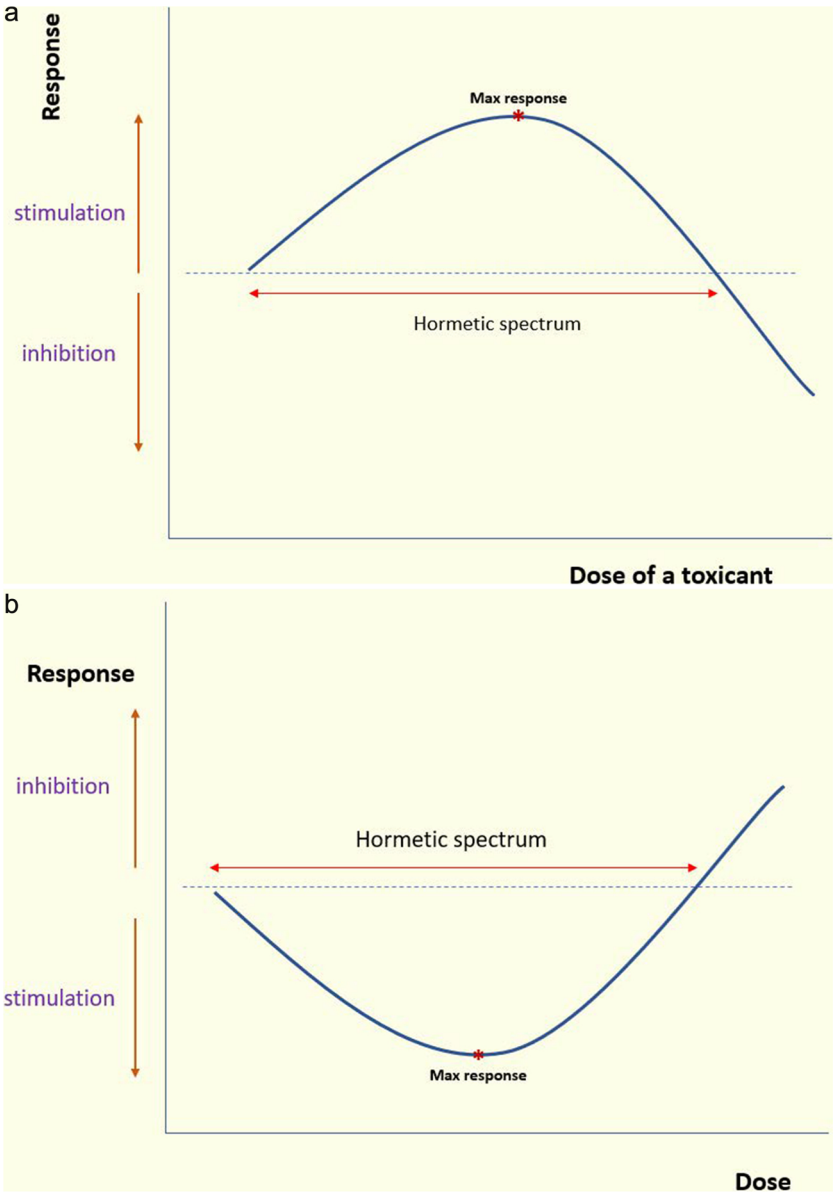

Fig. 4. a & b: Dose–response curve depicting the quantitative feature of hormesis. The U-shaped response corresponds to elevation of a negative phenomenon incidence, while reduction is evident at lower doses. On the other hand, the inverted U-shaped curve depicts the enhancement in the incidence of a positive phenomenon when exposure to low doses occurs, but reduction can be seen at increased doses.

compensatory mechanism in response to disrupted homeostasis, all the while forming a subgroup of the broader notion of biphasic dose-response nature, which, in turn, also exhibits diverse mechanistic functions [29]. Adrenergic, dopamine, neuropeptides, serotonin, bradykinin and many other receptor systems that have been investigated, exhibit biphasic dose-responses through mechanisms regulated by various agonists, probably implying that the hormetic curves are, under such conditions, applicable. It is known that several factors involved in immunity and tumorigenesis exhibit hormetic biphasic dose response patterns [30]. Agents that display hormetic behavior through a known receptor and/or a cellular signaling mechanism in terms of dose response relation include various endogenous agonists, including among others estradiol, angiotensin II, histamine, corticosteroids, serotonin, TGF-β, TNF-a, growth hormone, progesterone, oxytocin, vitamin D3, and toxins/drugs such as opiate agonists, organochlorine compounds, nicotine and pyrethroids, as well as atorvastatin, fluoxetine and morphine [31]. It has been observed that hormesis can be also applied in the environment of mitochondrial function. Mitochondrial hormesis is a phenomenon, where decreased ROS levels induce elevated mitochondrial function, while high levels cause lower performance [32]. Oxidative stress processes demonstrate a hormetic performance in the renal environment as well [33]. Moreover, hormesis has been shown to be applied also in the action of amyloid-β protein in memory capacity [34] and in ionizing radiation [35].

Hormesis functions as an adaptive response that can either be directly provoked or be induced as an overcompensation outcome, subsequently to homeostasis modifications [36]. Herein, it is important to note that, despite direct stimulation and stimulation as overcompensation, the ultimate tendency is to solidify homeostasis. Toxicity occurs when the homeostatic mechanisms are unable to reinstate the system disruption, due to overload of factors that act additively in damage and need to be compensated by the system through increased counteractive work.

Hormesis also exhibits optimality. Apart from being a tool for compensating disrupted homeostasis, hormesis can be deemed a way of optimization a response sequence that guarantees the attainment of homeostasis rebound. This can be performed by correcting the malfunction in a modestly surplus way, thus achieving not only the fixation of the lesion, but also the provision of an adequate mechanism that would protect the system from possible damage in the near future (approximate duration of several days), caused by a subsequent exposure [27].

What is evident is that hormesis seems to be frequently encountered. Studies support the beneficial use of the hormetic phenomenon in risk assessment for regulatory purposes [30], while other studies postulate a different approach (namely attention when naming a dose response hormetic) [37]. The classical dose-response approach has been reported to be insufficient to assess the toxic potency of some chemicals. One approach may be high-throughput screening assays for estimating the probability for chemicals to induce adverse effects, via a Bayesian hierarchical model called ZIPLL [38].

The need for a concise definition of hormesis still persists [16]. In any case, it has been suggested by Calabrese and his colleagues that assimilation of the hormesis model into the risk assessment process would bring more solid results, whether the response to low-dose exposure is harmful or beneficial [22]. However, naming a dose response relationship as hormetic in an attempt to justify the exposure outcome without adequate data might prove to be precarious to public health strategies [39].

2. Discussion

The significance of the right dose has been recognized for centuries. These adages warn of the harmful consequences of excess. According to Hesiod, absence of moderation causes *hybris*, thus leading to the divine retribution of Nemesis. In the 16th century Paracelsus extended the maxims of moderation to the context of drugs and poisons. «*Alle Dinge sind Gift und nichts ist ohne Gift, allein die Dosis macht es, dass ein Ding kein Gift ist*» (All things are poison and nothing is without poison, only the dose permits something not to be poison). Paracelsus recognized that toxicity occurred due to specific chemicals, and the body's response to the chemicals depended on the dose. Paracelsus reasoned that therapeutic agents can be harmful at high doses, and conversely substances considered toxic can be less harmful or even beneficial at lower doses. His understanding of dose and effect has been paraphrased in the statement "The dose makes the poison".

Knowledge of the dose response relationship enables a better understanding of causality between a toxicant and its effects, the minimum dose where an affect appears, and the rate of manifestation of the adverse effect. The nature of the dose response and its accompanying mechanism(s) remain an issue in toxicology. The risk assessment, management and communication are frequently affected by incertitude, as the toxic effects of exposure can be easily underestimated, especially when adequate information is lacking.

Nowadays, the experts in the field of toxicology after many years of research have captured the "essence" of dose-response concept especially in the vast majority of human real-life exposure scenarios where we can found two main key components, low doses and many contributing chemicals that create a final effect [40,41]. In regulated chemicals, safe exposure limits are set based on a) studies of single chemicals administered at high doses, b) the no-observed-adverse-effect-level (NOAEL) assuming by default monotonicity and high adversity, and c) using non-validated uncertainty factors, common for all chemicals.

Safe doses for a variety of chemicals in humans have been legally set by the European Union, the United States and a number of other governments; however, biomonitoring studies have demonstrated an association between exposure to mixtures of such chemicals even at these "safe levels" with adverse health outcomes [40,41].

It has been observed that exposure to organophosphorus and organochlorine pesticides in subclinical doses over long periods of time can result in adverse effects in exposed populations [42]. Specifically for pesticide risk assessment, biological and environmental monitoring are key elements; however, in agriculture, due to the open-environment, such monitoring is often difficult [43]. Thus, it is required that studies of single chemicals should be addressed with other aspects introducing determination of real-life exposure scenarios, hazard after real-life exposure scenarios, safety limits after real-life exposure scenarios, the use of new tools such as MoA/AOP, in silico, in vitro, and -omics for the hazard assessment and for the refinement of final in vivo studies.

In the case of human toxicology, researchers face a particular situation in which the same people might have been exposed to a multitude of chemicals through the environment, from use of consumer products or at the workplace (occupational exposure) and many times simultaneously or sequentially. This makes exposure assessment even more complicated and hard to identify linkage with long-term adverse health effects. Since human behavior shows an appreciable degree of variety, exposure will also vary greatly over the heterogeneous population, which differs with the genetically homogenous, inbred strains of experimental animals where regulatory toxicology studies are performed. Therefore, a number of exposure scenarios are needed, and the choice of exposure scenario will have a major influence on the results of an exposure assessment. So, what is the relation of the current toxicological approaches used for human (and environmental) protection with the real-life exposure scenarios and the actual hazard under such scenarios? This question will be addressed in the current special issue.

The challenge of cumulative risk-assessment could be reversed through the integration of toxicology, exposure assessment and epidemiology [44]. An important part of this integration is a better understanding of endocrine disruption, mechanistic pathways and target-organ toxicity [41].

The acceptable daily intakes and tolerable daily intakes have set

their basis in conventional risk assessment, which is oriented towards the afore-mentioned pattern of 'one-exposure-for-one-health-effect'. Thus, the true extent of exposure is often underestimated. In a study by Tsatsakis et al. [45], the assessment of toxic effects in individuals exposed to low doses of chemicals, was undertaken by studying simultaneously different endpoints (hormonal levels, neurotoxic factors, oxidative stress, hepatic enzymes, hematological biomarkers and histopathological evaluation). A worth-pointing limitation, however, is the incertitude due to toxicodynamic interactions occurring between chemicals; synergism as well as antagonism can develop among chemicals comprising the mixture, thus altering the expected outcome. Studies, such as these, could play a critical role in the elucidation of the causation of chronic ailments. Toxicology should be fortified in the academic environment, as it represents a necessity for the protection of public health and the environment.

Conflict of interest

None.

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Competing interests

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