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Towards a toxic-free environment: perspectives for chemical risk assessment approaches.

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Abstract

Regulatory frameworks to control chemical exposure in general living and occupational environments have changed exposure scenarios towards a widely spread contamination at relatively low doses in developed countries. In such evolving context, some critical aspects should be considered to update risk assessment and management strategies. Risk assessment in low-dose chemical exposure scenarios should take advantage of: toxicological investigations on emerging substances of interest, like those recognised as endocrine disruptors or increasingly employed nanoscale materials; human biological monitoring studies aimed to identify innovative biomarkers for known chemical exposure; "omic" technologies useful to identify hazards of chemicals and their modes of action. For updated risk assessment models, suitable toxicological studies, analyses of dose-responses at low-concentrations, environmental and biological monitoring of exposure, together with exposome studies, and the proper definition of susceptible populations may all provide helpful contributions. These may guide defining preventive measures to control the exposure and develop safe and sustainable chemicals by design. Occupational medicine can offer know-how and instruments to understand and manage such evolution towards a toxic-free environment to protect the safety and health of the workforce and, in turn, that of the general population.

Introduction

Chemicals have a key role in most of our activities and are ubiquitous in our daily life as they form part of almost every device we employ (1). World chemicals sales was valued at € 3,669 billion in 2019 and, with 14.8%, Europe was the second largest chemicals producer in the world in that year, following China, with the United States coming in third with 13.8% (2). The EU base chemical manufacturing supplies the 59% of chemicals to other sectors, such as constructions, electronics, automotive, textiles and health (3). This large chemical production and application inevitably cause environmental and human exposure, both in general living and occupational environments, raising concerns on possible

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adverse health effects. In this scenario, dangerous substances continue to be a major safety and health issue in workplaces (4). Certain chemicals, in fact, can cause cancers, affect the immune, respiratory, endocrine, reproductive and cardiovascular systems and increase vulnerability to diseases.

In the last few decades, this has led the adoption of comprehensive and protective regulatory frameworks for chemicals aimed to reduce the risks to humans and the environment, to provide predictable legislative framework for companies to operate in, while ensuring an efficient market for chemicals. These were primarily based on the avoidance of exposure sources, the elimination as far as possible of substances of concern for non-essential uses as well as in waste and secondary raw materials, and strict regulation based on risk assessment (5). This is in line with the vision to achieve a toxic-free, zero-pollution environment in the long-term, with safe and sustainable by design chemicals enabling the green and digital transitions of the chemical industry for the protection of the environment and human health (3).

These ambitious aims raise questions concerning the effectiveness of currently adopted approaches and policies to manage chemical risks and whether additional efforts should be planned in keeping with the dynamics of shifting pollution patterns for both the general and occupationally exposed populations. In fact, today's chemical exposures are characterised by a widely spread blanket of contamination composed of myriads of chemicals, all at rather low levels (6). These may include multiple pesticide residues, heavy metals, polychlorinated dioxins, furans and biphenyls, polybrominated and polyfluorinated compounds, polycyclic aromatic hydrocarbons, phthalates and other chemicals (6).

In this view, as the starting and key point to risk reduction and prevention is risk assessment, our aim was to provide some critical reflections focused at defining how specific strategies adopted to assess, and consequently manage, chemical risks in public and occupational settings should be revised in such evolving exposure scenarios. Therefore, we retraced the theoretical foundation to achieve a suitable risk assessment process pointing out some possible improvements in terms of exposure assessment, identification of early biological effects, as well as employment of innovative technologies, and concluded with a discussion on the implications that these issues may have for chemical risk management.

Exposure assessment

In the framework of human health risk assessment of chemicals, the default approach is either to consider only external exposure or to infer internal exposure from the external concentration measurements by modelling (7). However, some uncertainties may characterise this methodology due to the possible overestimation or underestimation of the real internal exposure, especially when this can occur from different sources and through variable routes (7). To overcome such limitations, biological monitoring has been largely employed to assess the overall exposure to a variety of hazardous substances (8). In this regard, to be useful, biomarkers should be as specific as possible to the chemical agents and highly sensitive, so to detect even low levels of exposure (9-11).

To define relevant analytes is a quite challenging issue. This may be the case of Chromium (Cr) exposure. In fact, although the total Cr in urine can be regarded as a reliable and sufficiently sensitive biomarker of internal dose, it does not offer a sufficient specificity to distinguish exposures to different trivalent Cr (Cr(III)) and hexavalent Cr (Cr(VI)) compounds that present diverse toxicokinetic and dynamic profiles (12). This prevents biomonitoring results to inform a suitable risk assessment and makes necessary to identify more specific biomarkers for Cr(VI) exposure in different biological matrices. These may include Cr in red blood cells (RBCs), as Cr(VI) can more easily permeate through the RBC membranes with respect to Cr(III) (13), and Cr in exhaled breath condensate (EBC), a biomarker able to provide specific information on the Cr(VI) levels in the main target organ, the lung (14).

Pioneering studies, in this regard, demonstrated that total Cr (15, 16) and Cr(VI) (16, 17) were measurable in the EBC of Cr plating workers and closely correlated (16). Interestingly, the fractional contribution of Cr(VI) to total Cr decreased from the last exposure in a time dependent manner, suggesting an interaction between inhaled Cr(VI) and

the pulmonary lining fluid, with a consequent, partial, reduction to Cr(III) (17). Overall, this supports the need to better understand Cr kinetics in the airways, considering also the solubility of the Cr compounds and the individual Cr(VI) reducing capability (16), and to assess the relationship between persistent Cr(VI) in the EBC and biomarkers of early effect (e.g. inflammatory and oxidative stress response) and pulmonary toxicity (17). This also suggests the relevance to better understand how the reduction kinetics of Cr(VI) in the EBC relate with and may be useful to monitor lung pathobiology. More recently, the EBC-total Cr was demonstrated as a reliable marker of exposure in stainless steel tungsten inert gas welders, although its weak relationship with the EBC biomarkers of oxidative stress suggests the need to deeply verify the complementary influencing role of other factors generated during the welding operations in determining biological alterations (18).Data from non-occupationally exposed populations, particularly the levels of Cr detected in the EBC of non-small cell lung cancer patients after surgical interventions, maybe related to the release of the metal from surgical instruments (19) and the presence of toxic elements, i.e. Lead, Cadmium and Aluminum, in patients with chronic obstructive pulmonary disease and asthma, as well as in tobacco smokers (20), further support the EBC-Cr as a suitable representative biomarker of exposure at the target organ level (19).

Additionally, in understanding a low-dose-response relationship, it is important to clarify whether possible non-linear, hormetic phenomena may occur. In the case of the Cr impact on the growth and development of alfalfa plants, lower Cr(VI) exposure (0.5 mg/L $K_2Cr_2O_7$) induced hormesis, as demonstrated by an increase in biomass and larger leaves, while opposite results were determined by higher concentrations (5 and 10 mg/L $K_2Cr_2O_7$) (21). As another example, when 4 human epidermal keratinocyte strains were treated with a metal mixture of arsenic, cadmium, Cr, and lead, cytotoxic effects were highly dose-dependent. A growth stimulatory effect (hormesis) was observed with the metal mixture at low concentrations (low ppb range), while as the mixture concentration increased, a trend of additivity changed to synergistic cytotoxicity in all 4 cell strains (22). Considering the essentiality of Cr in human beings, such possible dose response relationship should be further verified at low levels of occupational or general living exposure. Additionally, those molecular mechanisms underlying such possible kind of response should be clarified, in order to define whether the deficiency of the metal or its possible forms and physico-chemical features can play a role in determining a different bioavailability and toxicokinetics of the compounds and, consequently, a diverse dose-response and toxicological profile.

The exposure of humans, and particularly workers, to endocrine disrupting chemicals requires specific investigation. Among those, the ubiquitous occurrence of phthalates, substances added to plastics to increase their flexibility, durability and longevity, heat resistance and electrical resistivity, has raised great scientific attention. In most commercial products phthalates are used as additives, and they easily migrate from those products into the environment and food through evaporation, leaching and abrasion. Phthalates have been measured in a range of environmental matrices, including air, dust, soil, water, sludge, as well as in food. Indeed, individuals are unavoidably and regularly exposed to phthalates via multiple sources and pathways, as also demonstrated by the biomonitoring data of phthalates in the general worldwide population (23). This underlines the importance of human biomonitoring in defining the internal exposure to such chemicals. However, an understanding of various phthalates i.e. the old ones (whose employment was restricted due to their known endocrine disruptive properties) and newer ones, and their toxico-kinetics and dynamics, is important to identify valuable biomarkers of exposure. A recent review, in this regard, pointed out that most of occupational human biomonitoring studies analysed old phthalates, in plastics production fields, using not always appropriate metabolites (23). Moreover, future research should be focused on assessing phthalate exposure in different occupational sectors expected to include a large number of workers, such as waste management and recycling, or other possible fields of newer phthalate application (i.e. construction sectors, production of waterproof

gloves, tablecloths and floor tiles). These new biomonitoring data will result in evidence that may be useful to prioritise actions and measures for policymaking, evaluate the effectiveness of the policy measures adopted and promote more comprehensive health impact assessments of management options (23). Bisphenol A (BPA) and its substitutes bisphenol S (BPS) and bisphenol F (BPF) are endocrine disrupting chemicals widely used in the production of polycarbonate plastics, epoxy resins and thermal papers (24). However, a small number of human biomonitoring occupational exposure studies are worldwide available, especially on BPS and BPF, bisphenol analogues adopted as "safer" alternatives to BPA, whose toxicological profile is, unfortunately, still not completely understood. Therefore, further human biomonitoring investigation is necessary and should address also additional workplace settings, including those where plastic, epoxy resin and BPA-filled wax are manufactured and used (i.e. highest exposure industries).

To have more specific information on human exposure can be also useful to understand the reliability of biomarkers in different general living or occupational exposure conditions and to refine risk assessment and management processes (25, 26). In fact, data generated by the rapidly evolving human biomonitoring programs are providing invaluable opportunities to support and advance regulatory risk assessment and management of chemicals in occupational and environmental health domains (27). As previously noted for Cr, the selection of the proper biomarkers for the biomonitoring programs is important. Similarly, the strategy used to collect samples for biomonitoring needs to be carefully developed and also the performance of the laboratory cannot be overstated. However, to date, heterogeneity across studies, in terms of design, terminology, biomarker nomenclature, and data formats, limits comparison and integrations of data sets retrospectively. In this view, future approaches should be focused on standardizing biomonitoring strategies, such as procedures to assure sample traceability, proper collection of samples (including the definition of appropriate timing for sampling, especially for chemicals with a short half-life, like phthalates and bisphenols), as well as standard operating procedures for sample storage and transfer in order to obtain comparable data between countries and different investigations (25).

Concerning innovative materials, the widespread application of nano-enabled products and the increasing likelihood for workplace exposures make understanding engineered nanomaterial (ENM) exposure and health effects a public health priority. However, despite the extensive production and use of nano-products, our knowledge concerning NM health and safety issues is still in a developing phase, and also the occupational risk assessment derived from NM exposure remains a challenging task (28). This is related to the difficulties in assessing NM exposure levels through both environmental and biological monitoring measures. Currently, in fact, there are no examples of human biomonitoring as a part of routine assessment of workers exposed to ENMs and no regulatory requirements are available for biological monitoring assessment (29, 30). Concerning internal doses in exposed subjects, preliminary and fragmented data are available concerning the determination of the Ti elemental metal content in the EBC of TiO_2-NM production workers (31) and ^{68}Zn in urine samples collected from healthy volunteers applied on the back skin with ^{68}ZnO-nanoparticle containing sunscreens (32, 33). Also, in airport employees, operating on the apron, nearby airplane parking positions, and exposed via inhalation to incidental ultrafine particles generated by jet engines (mean particle size of 17.7 nm), a sparse population of around 500 nm sized particles in the EBC samples could be determined and concentrations of metals, i.e. Aluminum, Cadmium and Chromium were detected in 19%, 22% and 79% of all subjects, respectively (34). Some serum and urinary biomarkers, assessed through metabolomics among workers occupationally exposed to TiO_2-nanoparticles, have been suggested to potentially function as indicators of NM exposure (35, 36). Additionally, genotoxic and oxidative effect biomarkers could represent useful tools for the biomonitoring of workers exposed to nanoparticles, although they need to be confirmed on a high number of subjects (37). However, up to date, no biomarkers have been used in risk assessment processes. In this scenario, an exposure-response modeling should be pursued

to fully exploit the potential of possible exposure indicators. In fact, when the dose-response relationship is defined, the biomarker of exposure does not only indicate the dose actually adsorbed but provides also a reasonably accurate quantitative estimate of the occupational risks at the group and/or individual level (38).

Overall, the concept of the exposome, which encompasses all exposures over a lifetime, has the potential to improve chemical risk assessment (39-41). The exposome will rely on high throughput techniques for the identification of biomarkers of exposure. The exposome has the potential to offer more comprehensive exposure data that can be used to develop more accurate exposure profiles to improve risk assessment. It is recognised as a major conceptual advancement in environmental epidemiology, and there is an increased demand for technologies that can capture the spatial, temporal, and chemical variability of exposures across individuals (42). However, some "information biases" may affect the employment of such exposome approach. These may include the possibility for an overestimation of the exposure to persistent organic substances, as well as difficulties in measuring exposures to such compounds that are quickly metabolised (e.g., phthalates and organophosphate esters) or in taking into account the variability in individuals' systemic absorption and metabolism of chemicals that can influence the health impact of the exposure (43).

Biomarkers of effect

In addition to the exposure biomarkers, the identification of suitable biomarkers of effect is of outmost importance to link the exposure to the potential impact on human health. Traditional toxicology is rapidly moving towards a system-based approach. This can offer the opportunity to determine almost all the interactions between endogenous and/or exogenous xenobiotics and living systems, particularly as concerns how chemicals can affect normal biological processes, including homeostatic and adaptive responses, but also pathways leading to biological alterations (44). This methodology would provide integrated information that could be used in delineating the mechanisms underlying adverse response or toxicity, thus supporting changes in health risk assessment methods, including the setting of occupational exposure limits (OELs) (45). In fact, a better understanding of biological responses at lower levels of chemical exposure will have practical long-term implications, including the reduction in OEL uncertainties (46).

In this view, "omic" technologies offer the opportunity to better identify the hazardous properties of the xenobiotics, to investigate adverse responses, underlying toxicity mechanisms and pathways, as well as to develop novel biomarkers of exposure and early effect (46-48). Genomic, proteomic, metabolomic changes, can be useful to delineate genetic-based differences in chemical toxicokinetics that, in turn, might explain biomonitoring variabilities, potentially representing conditions of hyper-susceptibility to adverse health effects. High-performance omic technologies, together with the rate at which biomarker candidates will be discovered, will allow the use of a combination of biomarkers for a more precise selection of subjects with different outcomes and responses to chemical exposures (49). However, although innovative biomarkers can be identified using -omic technologies, there is no well-established standardised application of these biomarkers in risk assessment strategies, an issue that needs to be focused in future research (46). Additionally, also biomarkers of epigenetic alterations, can be indicative of early alterations and provide information on the molecular pathways of the chemical action, being able to explain individual differences in the toxicokinetics and toxicodynamics of the substance and variations in dose-response relationships.

Preliminary applications of omic technologies into occupational health fields have been described (50). In the case of solvent exposure, the analysis of the toxicogenomic and epigenomic profiles has revealed useful to better understand the mechanisms by which benzene may cause leukemia (51, 52). Occupational susceptibility to benzene hematotoxicity in exposed workers could be influenced by genetic variants in benzene key metabolising enzymes (53), but also by polymorphisms in genes

involved in DNA doublestrand breaks repair and genomic maintenance (54, 55). Proteomic analysis revealed that protein profiles were significantly different in benzene exposed workers compared to controls (56). As regards metals and metalloids, different genome expression patterns were detected in subjects with and without arsenical skin lesions (57) and also different proteomic signature was evident in smelter workers with a mixed exposure to arsenic and lead (52). A significant dose-dependent DNA hypermethylation was observed in arsenic-exposed people compared to controls (58). Also in the case of formaldehyde (FA), a general living and occupational pollutant, well known for its genotoxic and carcinogenic properties, a potential contribution of epigenetic effects cannot be excluded. DNA global methylation changes were demonstrated in workers exposed to formaldehyde FA and also epigenetic alterations were reported in in vitro and in vivo experiments (59, 60). Overall, although preliminary, these findings suggest the role of epigenetic modifications as possible underlying chemical mechanisms of action that need deeper qualitative and quantitative investigation. This may provide indicators of early biological effects and should be included in future risk assessment and management strategies for generally and occupationally exposed populations.

These innovative technologies may support a personalised approach to the hazard identification phase of risk assessment to include, not only the intrinsic toxicological profile of substances, but also how these may interact with the organisms. Additionally, this may offer the opportunity to generate comprehensive toxicologically relevant information on molecular changes more quickly and more accurately than ever before, supporting the identification of new hazards through enhanced coverage of biological or biochemical pathways during toxicological analyses (61). This approach might be even more important to support suitable risk assessment in emerging occupational scenarios, characterised by low-doses of exposure, employment of innovative materials (like chemicals at the nanoscale), as well as in settings where complex mixtures are used.

Biomarkers in the low-exposures scenario: examples and opportunities

Airborne benzene exposure in downtown Milan

Benzene is an established human carcinogen (62) that, due to its multiple sources of emission, is widely distributed both in occupational and in environmental settings. Airborne benzene levels had progressively decreased in traditionally polluted working settings but, as a component of engine emissions, remains an important pollutant in largest cities settings. Moreover, benzene is an important component of cigarette smoke (63). Consequently, benzene exposure in living and working environment is often very similar. This needs the definition of suitable, specific and sensitive biomarkers of exposure, that not necessarily are the one validated in previous occupational setting characterised by highest levels of exposure. In this regard, a study (64) compared urinary *trans, trans*-muconic acid (t,t-MA), S-phenylmercapturic acid and benzene (u-benzene) as biomarkers in gas station attendants (median benzene exposure 61 $\mu g/m^3$), urban policemen exposed to traffic emissions (median 22 $\mu g/m^3$), and blue-collar controls working in Milan downtown (6 $\mu g/m^3$). U-benzene, but not t,t-MA and S-phenylmercapturic acid, showed an expose-related increase. Moreover, all biomarkers were sensibly increased in smokers with u-benzene that resulted the best biomarker in distinguishing smoke-related exposure in such scenario.

The importance of a proper biomonitoring and the need of suitable markers is further supported by the evidence described by Bollati et al (65) that, even with these very low levels of exposure, workers showed a dose dependent reduction in global methylation (measured in LINE-1 and alu repetitive elements) and a gene-specific hypermethylation (p15). These patterns of altered methylation reproduce the epigenetic changes found in malignant cells (66).

Metal-rich fine particles effect among steel workers

Another example of hazard identification and evaluation of possible mechanism of actions based

on new biomarkers of effect comes from studies conducted among steel workers still exposed to high levels of metal-rich fine particles (67). A group of 63 male steel workers has been characterised in term of fine and coarse particle exposure calculated as the average of area-specific PM levels weighted by the time spent in each area (68). Airborne concentrations of individual metal particle components in PM_{10}, was calculated in each working area of the plant through multi-elemental analysis performed by means of inductively coupled-plasma mass spectrometer (ICP-MS). For each subject, blood samples have been collected on two different days: on the first day of a workweek (Day 1, following two days off work) and after three days of work (Day 4). Both global methylation and gene specific methylation resulted associated with PM exposure and with some of its metal components. Moreover, fine particles, Iron and Zinc exposure resulted significantly associated with histones modification (in details increased extracellular H3K4 methylation and H3K9 acetylation, (69)). PM1, Zinc and Iron resulted also associated to important coagulation parameters: Tissue plasminogen activator (t-PA) and Endogenous Thrombin Potential (ETP-defined as the amount of thrombin that is generated in plasma in vitro and has been proposed as a candidate test to reflect hypercoagulability). Further, increasing levels of exposure to coarse or ultra-fine PM were associated with shorter Prothrombin time, increased ETP, t-PA and C Reactive Protein (CRP). These results show that PM exposure increases coagulation function, and indicate that PM-induced systemic inflammation, as also reflected in increased plasma CRP, may enhance blood coagulation by tissue-factor triggering of the extrinsic coagulation pathway. These findings give further support to the hypothesis that inflammation-related hypercoagulability may mediate PM cardiovascular effects in exposed subjects (68).

Conclusions

Humans and the environment are widely exposed to low levels of different chemicals that require updated strategies for risk assessment and management. Additionally, in this latter decade, concerns about the combined effects of chemicals have been growing. This is due to the fact that the current risk assessment pathways have been generally based on single components, not specifically addressing the low-dose exposures (70), and not regulatory frameworks have been established for mixtures (71). In this view, to face the challenges posed by low-dose and mixture exposures, suitable toxicity studies should be pursued, based on adequate in silico, in vitro or, where necessary, in vivo experiments (72-75). In vitro studies could be helpful to predict the hazard of individual compounds and their combinations, as well as to define substances' modes of action at low doses, also as concerns possible concentration additivity. In silico and in vivo studies can further support the assessment of chemical risk in terms of internal exposure concentrations, but also in defining simultaneous or sequential exposure to different mixture components and to predict their potential toxicokinetic interactions. In this context, the use of physiologically based kinetic modeling may be important to interpret human biomonitoring data in toxicological risk assessment.

Omic techniques can additionally support the assessment of affected pathways for unraveling modes of action, as well as to elucidate possible antagonistic or synergistic interactions between substances (76). Concerning the dose–response relationships, toxicological studies should include a range of doses to distinguish between linear monotonic and non-monotonic responses, that should be carefully considered (5, 77, 78).

All this information may support modeling frameworks for defining "adverse outcome pathways" aimed to integrate and interpret hazard data in order to understand the continuum between a molecular initiating event and adverse effects (79, 80). In low-dose and mixture exposure scenarios, where to establish a causal relationship between the exposure and health effects represents an even more challenging issue, to identify possible adverse outcome pathways may be extremely useful to overcome these gaps, particularly from an occupational health perspective. Risk assessment and management approaches may be supported by such frameworks that could allow to group emerging exposures according to the chemicals' mechanisms of action,

structure (for which a similar toxicity profile is expected), common target organs or adverse outcomes (81). All these elements may provide an orientation to these strategies especially when emerging substances, whose toxicological profile is not fully explored, are considered. Moreover, updated risk assessment processes, should integrate toxicological data with information on the currently experienced conditions of exposure. Target population(s), sources and routes of exposure, doses and temporal aspects (i.e. episodic or repeated, simultaneous or sequential exposures), along with the chemicals involved should be addressed. All these features, in fact, may function as predictors for variability in toxicological behaviors (82). According to the toxicokinetic properties of chemicals, an external sequential co-exposure can result in an internal simultaneous co-exposure, and vice versa (83). Moreover, on a longer timescale, exposure to bioaccumulative and persistent compounds, is particularly relevant, as they can remain in an organism even after cessation of (external) exposure, increasing the potential for (internal) co-exposure. Additionally, exposure assessment should be addressed according to a life-cycle perspective of substances and products, that could change risks posed to human health (84).

Unfortunately, only a limited number of chemicals can be covered by environmental and biological monitoring activities. Indeed, considering the huge number of substances and their possible combinations, there is a clear need to identify and prioritise substances, as well as mixtures of concern, in order to effectively act on them. Priority chemicals may include those that are expected to be present at concentrations close to their effect level, mixtures of chemicals thought to act on the same pathway; substances assumed to have no threshold or be very persistent (85). Additionally, monitoring data should be generated, collected, storaged and used according to a more coherent and internationally shared approach (86). Furthermore, particularly in occupational settings, the link between chemical exposure and the human health status is often difficult to establish because of other non-chemical stress factors. Where applicable, the influence of other factors such as dietary status, habitat conditions, but also other occupational exposures should be also considered. In this view, one way to take a more holistic approach is to use exposome studies that look at a wider range of factors over longer periods (87-89).

The study of possible effects at low doses of exposure considers that the dose-response curve obtained in toxicity testing is a "picture" of the susceptibility distribution in the treated population (90). This understanding is particularly relevant for stochastic effects, i.e. genotoxic carcinogenesis, for which the assumption of a linear dose-response relationship at low dose is often adopted (91, 92). The exposure is scaled linearly by an effect factor representing a fixed slope of the dose-response relationship and homogeneous toxicological susceptibility to the chemical. This practice is based on the assumption that all individuals across a population share homogeneous exposure and identical heath response to a marginal increase in exposure (93). In addition, the acceptability of using a linear dose-response relationship in toxicity characterisation is also being questioned because it runs against our understanding of dose-response relationships for many substances. This opens some questions regarding appropriate risk management strategies (94, 95). Despite the above-detailed limitations, in clinical settings, the linear dose-response may represent the relationship between some pathological conditions and specific adverse outcomes. As an example, it is well known that among the hypertensive patients, a greater hypertension has a linear higher lifetime risk of stroke and efforts to adequately reduce blood (BP) pressure are mandatory (96). However, it remains to be clarified which is the optimal timing to initiate BP reduction and BP goals to be targeted with respect to a cost/benefit perspective (97). In a chemical exposure setting, to define a linear dose-response relationship may be challenging, although its identification is essential to plan the preventive and protective measures to adopt, the goals to achieve (in terms of exposure elimination/control) and the early effects to monitor.

Moreover, studies have long shown systematic inter-individual variabilities in human exposure and toxicological susceptibility due to a mixture of factors including genetic, development stage, dietary

pattern, behavior and pathological conditions (93). Therefore, the above-mentioned linear extrapolation model may leave the most susceptible individuals or sub-populations unprotected especially at low doses of exposure. In fact, these subjects would be underrepresented if generic, one-size-fits-all population characterisation factors, are employed, therefore preventing tailored, specifically focused risk assessment and management strategies. Furthermore, from a risk management perspective, it is essential to identify susceptible populations including pregnant women, elderly subjects and people affected by different kind of pathologies that may need targeted preventive actions, particularly in occupational settings (98). Precision, occupational medicine strategies, in this regard, may support such specific and personalised interventions (99). These may regard also the establishment of suitable guidance values, that should take into consideration not only the driving effect of chemicals, but also additional information on other types of effects that may be evident at lower doses. All the information retrieved through the above-mentioned investigations may provide guidance to the development of safe and sustainable by design chemicals and products that may characterise another interesting measure of chemical risk management.

Overall, the occupational medicine is absolutely engaged in understanding and actively manage such evolution towards a toxic free environment. This discipline can offer know-how and instruments to support the identification of the hazardous properties of different conditions of emerging exposure, to define suitable risk assessment strategies and preventive measures to protect the safety and health of the workforce and, in turn, that of the general population. However, collaborations between research scientists in academia, government, and industry should be encouraged to allow for development of more sophisticated study designs that may allow a deeper understanding of low-dose effects. Such concerted efforts may provide guidance to take appropriate actions to protect human and occupational health from harmful chemicals and facilitate better regulatory decision making.

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References

1. European Commission. 2019. The European Green Deal. Available at: https://eur-lex.europa.eu/resource.html?uri=cellar:b828d165-1c22-11ea-8c1f-01aa75ed71a1.0002.02/DOC_1&format=PDF (last accessed 23-12-2021).
2. CEFIC Chemdata International. 2021. Available on line at: https://cefic.org/a-pillar-of-the-european-economy/facts-and-figures-of-the-european-chemical-industry/profile/. (last accessed on 23-12- 2021).
3. European commission. 2020. Chemicals Strategy for Sustainability Towards a Toxic-Free Environment. Available online at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52020DC0667&from=EN (last accessed 23-12-2021).
4. EU-OSHA, 2018. Legislative framework on dangerous substances in workplaces. Available on line at: https://osha.europa.eu/lv/publications/info-sheet-legislative-framework-dangerous-substances-workplaces. (last accessed 23-12-2021).
5. Lee DH, Jacobs DR Jr. New approaches to cope with possible harms of low-dose environmental chemicals. J Epidemiol Community Health. 2019;73(3):193-197. doi: 10.1136/jech-2018-210920. Epub 2019 Jan 11. PMID: 30635437; PMCID: PMC6580748.
6. Kortenkamp A. Low dose mixture effects of endocrine disrupters and their implications for regulatory thresholds in chemical risk assessment. Curr Opin Pharmacol. 2014 ;19:105-11. doi: 10.1016/j.coph.2014.08.006. Epub 2014 Sep 19. PMID: 25244397.
7. Louro H, Heinälä M, Bessems J, Buekers J, Vermeire T, Woutersen M, van Engelen J, Borges T, Rousselle C, Ou-gier E, Alvito P, Martins C, Assunção R, Silva MJ, Pronk A, Schaddelee-Scholten B, Del Carmen Gonzalez M, de Alba M, Castaño A, Viegas S, Humar-Juric T, Kononenko L, Lampen A, Vinggaard AM, Schoeters G, Kolos-sa-Gehring M, Santonen T. Human biomonitoring in health risk assessment in Europe: Current practices and recommendations for the future. Int J Hyg Environ Health. 2019;222(5):727-737. doi: 10.1016/j.ijheh.2019.05.009. Epub 2019 Jun 5. PMID: 31176761.
8. Albertini R, Bird M, Doerrer N, Needham L, Robison S, Sheldon L, Zenick H. The use of biomonitoring data in exposure and human health risk assessments. Environ Health Perspect. 2006 Nov;114(11):1755-62. doi: 10.1289/ehp.9056.
9. Jakubowski M, Trzcinka-Ochocka M. Biological monitoring of exposure: trends and key developments. J Occup Health. 2005 ;47(1):22-48. doi: 10.1539/joh.47.22. PMID: 15703450.
10. Manno M, Viau C; in collaboration with, Cocker J,

Colosio C, Lowry L, Mutti A, Nordberg M, Wang S. Biomon-itoring for occupational health risk assessment (BOHRA). Toxicol Lett. 2010;192(1):3-16. doi: 10.1016/j.toxlet.2009.05.001. Epub 2009 May 13. PMID: 19446015.

11. Viegas S, Zare Jeddi M, Hopf NB, Bessems J, Palmen N, S Galea K, Jones K, Kujath P, Duca RC, Verhagen H, Santonen T, Pasanen-Kase R. Biomonitoring as an Underused Exposure Assessment Tool in Occupational Safety and Health Context-Challenges and Way Forward. Int J Environ Res Public Health. 2020;17(16):5884. doi: 10.3390/ijerph17165884. PMID: 32823696; PMCID: PMC7460384.
12. SCOEL/OPIN/2016-086 Chromium(VI) compounds. Opinion from the Scientific Committee on Occupational Exposure Limits. Available on line at: https://op.europa.eu/en/publication-detail/-/publication/bc28ab1d-c35f-11e6-a6db-01aa75ed71a1/language-en/format-PDF/source-search. (last accessed 23-12-2021).
13. Ray RR. Adverse hematological effects of hexavalent chromium: an overview. Interdiscip Toxicol. 2016;9(2):55-65. doi: 10.1515/intox-2016-0007. Epub 2017 May 17. PMID: 28652847; PMCID: PMC5458105.
14. Verdonck J, Duca RC, Galea KS Iavicoli I, Poels K, Töreyin ZN, Vanoirbeek J, Godderis L. Systematic review of biomonitoring data on occupational exposure to hexavalent chromium. Int J Hyg Environ Health. 2021;236:113799. doi: 10.1016/j.ijheh.2021.113799. Epub 2021 Jul 22. PMID: 34303131.
15. Caglieri A, Goldoni M, Acampa O, Andreoli R, Vettori MV, Corradi M, Apostoli P, Mutti A. The effect of in-haled chromium on different exhaled breath condensate biomarkers among chrome-plating workers. Environ Health Perspect. 2006;114(4):542-6. Doi: 10.1289/ehp.8506. PMID: 16581543; PMCID: PMC1440778.
16. Goldoni M, Caglieri A, De Palma G, Acampa O, Gergelova P, Corradi M, Apostoli P, Mutti A. Chromium in exhaled breath condensate (EBC), erythrocytes, plasma and urine in the biomonitoring of chrome-plating workers exposed to soluble Cr(VI). J Environ Monit. 2010;12(2):442-7. Doi: 10.1039/b914673c. Epub 2009 Nov 17. PMID: 20145884.
17. Goldoni M, Caglieri A, Poli D, Vettori MV, Corradi M, Apostoli P, Mutti A. Determination of hexavalent chromium in exhaled breath condensate and environmental air among chrome plating workers. Anal Chim Acta. 2006;562(2):229-235. Doi: 10.1016/j.aca.2006.01.065. PMID: 17047732; PMCID: PMC1615891.
18. Riccelli MG, Goldoni M, Andreoli R, Mozzoni P, Pinelli S, Alinovi R, Selis L, Mutti A, Corradi M. Biomarkers of exposure to stainless steel tungsten inert gas welding fumes and the effect of exposure on exhaled breath con-densate. Toxicol Lett. 2018;292:108-114. Doi: 10.1016/j.toxlet.2018.04.032. Epub 2018 Apr 30. PMID: 29719222.
19. Goldoni M, Caglieri A, Corradi M, Poli D, Rusca M, Carbognani P, Mutti A. Chromium in exhaled breath con-densate and pulmonary tissue of non-small cell lung cancer patients. Int Arch Occup Environ Health. 2008;81(4):487-93. Doi: 10.1007/s00420-007-0242-8. Epub 2007 Aug 28. PMID: 17724608.
20. Mutti A, Corradi M, Goldoni M, Vettori MV, Bernard A, Apostoli P. Exhaled metallic elements and serum pneumoproteins in asymptomatic smokers and patients with COPD or asthma. Chest. 2006;129(5):1288-97. Doi: 10.1378/chest.129.5.1288. PMID: 16685021; PMCID: PMC1472634.
21. Christou A, Georgiadou EC, Zissimos AM, Christoforou IC, Christofi C, Neocleous D, Dalias P, Torrado SOCA, Argyraki A, Fotopoulos V. Hexavalent chromium leads to differential hormetic or damaging effects in alfalfa (Medicago sativa L.) plants in a concentration-dependent manner by regulating nitro-oxidative and proline metabolism. Environ Pollut. 2020;267:115379. Doi: 10.1016/j.envpol.2020.115379. Epub 2020 Aug 20. PMID: 32841910.
22. Bae DS, Gennings C, Carter WH Jr, Yang RS, Campain JA. Toxicological interactions among arsenic, cadmium, chromium, and lead in human keratinocytes. Toxicol Sci. 2001;63(1):132-42. Doi: 10.1093/toxsci/63.1.132. PMID: 11509753.
23. Fréry N, Santonen T, Porras SP, Fucic A, Leso V, Bousoumah R, Duca RC, El Yamani M, Kolossa-Gehring M, Ndaw S, Viegas S, Iavicoli I. Biomonitoring of occupational exposure to phthalates: A systematic review. Int J Hyg Environ Health. 2020;229:113548. doi: 10.1016/j.ijheh.2020.113548. Epub 2020 Jul 11. PMID: 32659708.
24. Bousoumah R, Leso V, Iavicoli I, Huuskonen P, Viegas S, Porras SP, Santonen T, Frery N, Robert A, Ndaw S. Bi-omonitoring of occupational exposure to bisphenol A, bisphenol S and bisphenol F: A systematic review. Sci Total Environ. 2021;783:146905. doi: 10.1016/j.scitotenv.2021.146905. Epub 2021 Apr 5. PMID: 33865140.
25. Santonen T, Alimonti A, Bocca B, Duca RC, Galea KS, Godderis L, Göen T, Gomes B, Hanser O, Iavicoli I, Jana-sik B, Jones K, Kiilunen M, Koch HM, Leese E, Leso V, Louro H, Ndaw S, Porras SP, Robert A, Ruggieri F, Scheepers PTJ, Silva MJ, Viegas S, Wasowicz W, Castano A, Sepai O. Setting up a collaborative European hu-man biological monitoring study on occupational exposure to hexavalent chromium. Environ Res. 2019;177:108583. doi: 10.1016/j.envres.2019.108583. Epub 2019 Jul 10. PMID: 31330491.
26. Galea KS, Porras SP, Viegas S, Bocca B, Bousoumah R, Duca RC, Godderis L, Iavicoli I, Janasik B, Jones K, Knudsen LE, Leese E, Leso V, Louro H, Ndaw S, Ruggieri F, Sepai O, Scheepers PTJ, Silva MJ, Wasowicz W, San-tonen T. HBM4EU chromates study - Reflection and lessons learnt from designing and undertaking a collaborative European biomonitoring study on occupational exposure to hexavalent chromium. Int J Hyg Environ Health. 2021;234:113725. doi: 10.1016/j.ijheh.2021.113725.

Epub 2021 Mar 11. PMID: 33714856.
27. Zare Jeddi M, Virgolino A, Fantke P, Hopf NB, Galea KS, Remy S, Viegas S, Mustieles V, Fernandez MF, von Goetz N, Vicente JL, Slobodnik J, Rambaud L, Denys S, St-Amand A, Nakayama SF, Santonen T, Barouki R, Pasanen-Kase R, Mol HGJ, Vermeire T, Jones K, Silva MJ, Louro H, van der Voet H, Duca RC, Verhagen H, Ca-nova C, van Klaveren J, Kolossa-Gehring M, Bessems J. A human biomonitoring (HBM) Global Registry Framework: Further advancement of HBM research following the FAIR principles. Int J Hyg Environ Health. 2021;238:113826. doi: 10.1016/j.ijheh.2021.113826. Epub 2021 Sep 25. PMID: 34583227.
28. Leso V, Fontana L, Mauriello MC, Iavicoli I. Occupational Risk Assessment of Engineered Nanomaterials: Lim-its, Challenges and Opportunities. Current Nanoscience. 2017; 13: 55-78.
29. Schulte P, Leso V, Niang M, Iavicoli I. Biological monitoring of workers exposed to engineered nanomaterials. Toxicol Lett. 2018;298:112-124. doi: 10.1016/j.toxlet.2018.06.003. Epub 2018 Jun 18. PMID: 29920308; PMCID: PMC6239923.
30. Schulte PA, Leso V, Niang M, Iavicoli I. Current state of knowledge on the health effects of engineered nanomaterials in workers: a systematic review of human studies and epidemiological investigations. Scand J Work Environ Health. 2019;45(3):217-238. doi: 10.5271/sjweh.3800. Epub 2019 Jan 17. PMID: 30653633; PMCID: PMC6494687.
31. Pelclova D, Barosova H, Kukutschova J, Zdimal V, Navratil T, Fenclova Z, Vlckova S, Schwarz J, Zikova N, Kacer P, Komarc M, Belacek J, Zakharov S. Raman microspectroscopy of exhaled breath condensate and urine in workers exposed to fine and nano TiO2 particles: a cross-sectional study. J Breath Res. 2015;9(3):036008. doi: 10.1088/1752-7155/9/3/036008. PMID: 26172946.
32. Gulson B, McCall M, Korsch M, Gomez L, Casey P, Oytam Y, Taylor A, McCulloch M, Trotter J, Kinsley L, Greenoak G. Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin. Toxicol Sci. 2010;118(1):140-9. doi: 10.1093/toxsci/kfq243. Epub 2010 Aug 12. Erratum in: Toxicol Sci. 2011 ;120(2):530. PMID: 20705894.
33. Gulson B, Wong H, Korsch M, Gomez L, Casey P, McCall M, McCulloch M, Trotter J, Stauber J, Greenoak G. Comparison of dermal absorption of zinc from different sunscreen formulations and differing UV exposure based on stable isotope tracing. Sci Total Environ. 2012;420:313-8. doi: 10.1016/j.scitotenv.2011.12.046. Epub 2012 Feb 7. PMID: 22316633.
34. Marie-Desvergne C, Dubosson M, Touri L, Zimmermann E, Gaude-Môme M, Leclerc L, Durand C, Klerlein M, Molinari N, Vachier I, Chanez P, Mossuz VC. Assessment of nanoparticles and metal exposure of airport workers using exhaled breath condensate. J Breath Res. 2016;10(3):036006. doi: 10.1088/1752-7155/10/3/036006. PMID: 27409350.
35. Chen Z, Han S, Zhang J, Zheng P, Liu X, Zhang Y, Jia G. Exploring urine biomarkers of early health effects for occupational exposure to titanium dioxide nanoparticles using metabolomics. Nanoscale. 2021;13(7):4122-4132. doi: 10.1039/d0nr08792k. PMID: 33570056.
36. Chen Z, Han S, Zhang J, Zheng P, Liu X, Zhang Y, Jia G. Metabolomics screening of serum biomarkers for occu-pational exposure of titanium dioxide nanoparticles. Nanotoxicology. 2021;15(6):832-849. doi: 10.1080/17435390.2021.1921872. Epub 2021 May 7. PMID: 33961536.
37. Ursini CL, Fresegna AM, Ciervo A, Maiello R, Del Frate V, Folesani G, Galetti M, Poli D, Buresti G, Di Cristo L, Sabella S, Iavicoli S, Cavallo D. Occupational exposure to graphene and silica nanoparticles. Part II: pilot study to identify a panel of sensitive biomarkers of genotoxic, oxidative and inflammatory effects on suitable biological matrices. Nanotoxicology. 2021;15(2):223-237. doi: 10.1080/17435390.2020.1850903. Epub 2020 Dec 29. PMID: 33373530.
38. Iavicoli I, V. Leso, M. Manno, P.A. Schulte. Biomarkers of nanomaterial exposure and effect: current status. J Nanopart Res. 2014; 16:2302.
39. Wild CP. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev. 2005;14(8):1847-50. doi: 10.1158/1055-9965.EPI-05-0456. PMID: 16103423.
40. Huhn S, Escher BI, Krauss M, Scholz S, Hackermüller J, Altenburger R. Unravelling the chemical exposome in cohort studies: routes explored and steps to become comprehensive. Environ Sci Eur. 2021;33(1):17. doi: 10.1186/s12302-020-00444-0. Epub 2021 Feb 11. PMID: 33614387; PMCID: PMC7877320.
41. Vermeulen R, Schymanski EL, Barabási AL, Miller GW. The exposome and health: Where chemistry meets bi-ology. Science. 2020;367(6476):392-396. doi: 10.1126/science.aay3164. PMID: 31974245; PMCID: PMC7227413.
42. Doherty BT, Koelmel JP, Lin EZ, Romano ME, Godri Pollitt KJ. Use of Exposomic Methods Incorporating Sen-sors in Environmental Epidemiology. Curr Environ Health Rep. 2021;8(1):34-41. Doi: 10.1007/s40572-021-00306-8. Epub 2021 Feb 10. PMID: 33569731.
43. Bocato MZ, Bianchi Ximenez JP, Hoffmann C, Barbosa F. An overview of the current progress, challenges, and prospects of human biomonitoring and exposome studies. J Toxicol Environ Health B Crit Rev. 2019;22(5-6):131-156. doi: 10.1080/10937404.2019.1661588. Epub 2019 Sep 5. PMID: 31543064.
44. Edwards SW, Preston RJ. Systems biology and mode of action-based risk assessment. Toxicol Sci. 2008;106(2):312-8. doi: 10.1093/toxsci/kfn190. Epub 2008 Sep 12. PMID: 18791183.
45. McHale CM, Zhang L, Hubbard AE, Smith MT.

Toxicogenomic profiling of chemically exposed humans in risk assessment. Mutat Res. 2010;705(3):172-83. doi: 10.1016/j.mrrev.2010.04.001. Epub 2010 Apr 9. PMID: 20382258; PMCID: PMC2928857.

46. DeBord DG, Burgoon L, Edwards SW, Haber LT, Kanitz MH, Kuempel E, Thomas RS, Yucesoy B. Systems Biology and Biomarkers of Early Effects for Occupational Exposure Limit Setting. J Occup Environ Hyg. 2015;12 Suppl 1:S41-54. doi: 10.1080/15459624.2015.1060324. PMID: 26132979; PMCID: PMC4654673.
47. Schulte PA, Hauser JE. The use of biomarkers in occupational health research, practice, and policy. Toxicol Lett. 2012;213(1):91-9. doi: 10.1016/j.toxlet.2011.03.027. Epub 2011 Apr 6. PMID: 21477643.
48. Hernández-Mesa M, Le Bizec B, Dervilly G. Metabolomics in chemical risk analysis - A review. Anal Chim Acta. 2021;1154:338298. doi: 10.1016/j.aca.2021.338298. Epub 2021 Feb 17. PMID: 33736812.
49. Corradi M, Goldoni M, Mutti A. A review on airway biomarkers: exposure, effect and susceptibility. Expert Rev Respir Med. 2015;9(2):205-20. Doi: 10.1586/17476348.2015.1001373. Epub 2015 Jan 6. PMID: 25561087.
50. Vlaanderen J, Moore LE, Smith MT, Lan Q, Zhang L, Skibola CF, Rothman N, Vermeulen R. Application of OM-ICS technologies in occupational and environmental health research; current status and projections. Occup En-viron Med. 2010;67(2):136-43. doi: 10.1136/oem.2008.042788. Epub 2009 Nov 20. PMID: 19933307; PMCID: PMC2910417.
51. Smith MT, Vermeulen R, Li G, Zhang L, Lan Q, Hubbard AE, Forrest MS, McHale C, Zhao X, Gunn L, Shen M, Rappaport SM, Yin S, Chanock S, Rothman N. Use of 'Omic' technologies to study humans exposed to benzene. Chem Biol Interact. 2005;153-154:123-7. doi: 10.1016/j.cbi.2005.03.017. Epub 2005 Apr 19. PMID: 15935808.
52. Zhai R, Su S, Lu X, Liao R, Ge X, He M, Huang Y, Mai S, Lu X, Christiani D. Proteomic profiling in the sera of workers occupationally exposed to arsenic and lead: identification of potential biomarkers. Biometals. 2005;18(6):603-13. doi: 10.1007/s10534-005-3001-x. PMID: 16388400.
53. Lan Q, Zhang L, Li G, Vermeulen R, Weinberg RS, Dosemeci M, Rappaport SM, Shen M, Alter BP, Wu Y, Kopp W, Waidyanatha S, Rabkin C, Guo W, Chanock S, Hayes RB, Linet M, Kim S, Yin S, Rothman N, Smith MT. Hematotoxicity in workers exposed to low levels of benzene. Science. 2004;306(5702):1774-6. doi: 10.1126/science.1102443. PMID: 15576619; PMCID: PMC1256034.
54. Lan Q, Zhang L, Shen M, Jo WJ, Vermeulen R, Li G, Vulpe C, Lim S, Ren X, Rappaport SM, Berndt SI, Yeager M, Yuenger J, Hayes RB, Linet M, Yin S, Chanock S, Smith MT, Rothman N. Large-scale evaluation of candidate genes identifies associations between DNA repair and genomic maintenance and development of benzene hem-atotoxicity. Carcinogenesis. 2009; 30(1):50-8. doi: 10.1093/carcin/bgn249. Epub 2008 Oct 31. PMID: 18978339; PMCID: PMC2639030.
55. Shen M, Lan Q, Zhang L, Chanock S, Li G, Vermeulen R, Rappaport SM, Guo W, Hayes RB, Linet M, Yin S, Yeager M, Welch R, Forrest MS, Rothman N, Smith MT. Polymorphisms in genes involved in DNA dou-ble-strand break repair pathway and susceptibility to benzene-induced hematotoxicity. Carcinogenesis. 2006;27(10):2083-9. doi: 10.1093/carcin/bgl061. Epub 2006 May 25. PMID: 16728435.
56. Joo WA, Sul D, Lee DY, Lee E, Kim CW. Proteomic analysis of plasma proteins of workers exposed to benzene. Mutat Res. 2004;558(1-2):35-44. doi: 10.1016/j.mrgentox.2003.10.015. PMID: 15036117.
57. Wu MM, Chiou HY, Ho IC, Chen CJ, Lee TC. Gene expression of inflammatory molecules in circulating lymphocytes from arsenic-exposed human subjects. Environ Health Perspect. 2003;111(11):1429-38. doi: 10.1289/ehp.6396. PMID: 12928151; PMCID: PMC1241636.
58. Chanda S, Dasgupta UB, Guhamazumder D, Gupta M, Chaudhuri U, Lahiri S, Das S, Ghosh N, Chatterjee D. DNA hypermethylation of promoter of gene p53 and p16 in arsenic-exposed people with and without malignancy. Toxicol Sci. 2006 ;89(2):431-7. doi: 10.1093/toxsci/kfj030. Epub 2005 Oct 26. PMID: 16251483.
59. Chappell G, Pogribny IP, Guyton KZ, Rusyn I. Epigenetic alterations induced by genotoxic occupational and en-vironmental human chemical carcinogens: A systematic literature review. Mutat Res Rev. 2016;768:27-45. doi: 10.1016/j.mrrev.2016.03.004. Epub 2016 Mar 31. PMID: 27234561; PMCID: PMC4884606.
60. Leso V, Macrini MC, Russo F, Iavicoli I. Formaldehyde Exposure and Epigenetic Effects: A Systematic Review. Appl. Sci. 2020, 10, 2319.
61. Buesen R, Chorley BN, da Silva Lima B, Daston G, Deferme L, Ebbels T, Gant TW, Goetz A, Greally J, Gribaldo L, Hackermüller J, Hubesch B, Jennen D, Johnson K, Kanno J, Kauffmann HM, Laffont M, McMullen P, Meehan R, Pemberton M, Perdichizzi S, Piersma AH, Sauer UG, Schmidt K, Seitz H, Sumida K, Tollefsen KE, Tong W, Tralau T, van Ravenzwaay B, Weber RJM, Worth A, Yauk C, Poole A. Applying 'omics technologies in chemi-cals risk assessment: Report of an ECETOC workshop. Regul Toxicol Pharmacol. 2017;91 Suppl 1:S3-S13. doi: 10.1016/j.yrtph.2017.09.002. Epub 2017 Sep 25. PMID: 28958911; PMCID: PMC6816021.
62. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Benzene, Lyon (FR): International Agency for Research on Cancer; 2018.
63. Darral KG, Figgins JA, Brown RD, Philips GF Determination of benzene and associated volatile compounds in mainstream cigarette smoke, Analyst. 1998 ;123(5):1095-101. doi: 10.1039/a708664d.
64. Fustinoni S, Consonni D, Campo L, Buratti M, Colombi

A, Pesatori AC, Bonzini M, Bertazzi PA, Foà V, Garte S, Farmer PB, Levy LS, Pala M, Valerio F, Fontana V, Desideri A, Merlo DF Monitoring low benzene exposure: comparative evaluation of urinary biomarkers, influence of cigarette smoking, and genetic polymorphisms. Cancer Epidemiol Biomarkers Prev. 2005;14(9):2237-44. doi: 10.1158/1055-9965.EPI-04-0798.PMID: 16172237

65. Bollati V, Baccarelli A, Hou L, Bonzini M, Fustinoni S, Cavallo D, Byun HM, Jiang J, Marinelli B, Pesatori AC, Bertazzi PA, Yang AS. Changes in DNA methylation patterns in subjects exposed to low-dose benzene. Cancer Res. 2007;67(3):876-80. doi: 10.1158/0008-5472.CAN-06-2995. PMID: 17283117.
66. Chambost H, Brasseur F, Coulie P, de Plaen E, Stoppa AM, Baume D, Mannoni P, Boon T, Maraninchi D, Olive D. Share A tumour-associated antigen expression in human haematological malignancies. Br J Haematol. 1993;84(3):524-6. doi: 10.1111/j.1365-2141.1993.tb03111.x.PMID: 8217804.
67. Bertazzi PA, Bollati V, Bonzini M. (Hazard identification and risk evaluation in the metal industry: the epige-netic challenge) G Ital Med Lav Ergon. 2012 ;34(3):223-8. PMID: 23213793
68. Bonzini M, Tripodi A, Artoni A, Tarantini L, Marinelli B, Bertazzi PA, Apostoli P, Baccarelli A. Effects of in-halable particulate matter on blood coagulation. J Thromb Haemost. 2010r;8(4):662-8. doi: 10.1111/j.1538-7836.2009.03694.x. Epub 2009 Nov 17.PMID: 19922434
69. Cantone L, Angelici L, Bollati V, Bonzini M, Apostoli P, Tripodi A, Bertazzi PA, Baccarelli AA. Extracellular histones mediate the effects of metal-rich air particles on blood coagulation. Environ Res. 2014;132:76-82. doi: 10.1016/j.envres.2014.03.029. Epub 2014 Apr 16.
70. Calabrese EJ. Hormesis is central to toxicology, pharmacology and risk assessment. Hum Exp Toxicol. 2010;29(4):249-61.
71. Heys KA, Shore RF, Pereira MG, Jones KC, Martin FL. 2016. Risk assessment of environmental mixture effects. RSC Adv. 6:47844–47857.72. Altenburger R, Nendza M, Schüürmann G. Mixture toxicity and its modeling by quantitative structure-activity relationships. Environ Toxicol Chem. 2003; 22(8):1900-15. doi: 10.1897/01-386. PMID: 12924589.
72. Altenburger R, Scholz S, Schmitt-Jansen M, Busch W, Escher BI. Mixture toxicity revisited from a toxicogenomic perspective. Environ Sci Technol. 2012; 46(5):2508-22. doi: 10.1021/es2038036. Epub 2012 Feb 27. PMID: 22283441.
73. Bopp S, Berggren E, Kienzler A, van der Linden S, Worth A. 2015. Scientific methodologies for the assessment of combined effects of chemicals – a survey and literature review. JRC Technical Report. EUR 27471 EN, 64 pp. Publications Office of the European Union, Luxembourg. https://doi.org/10.2788/093511.
74. Kim J, Kim S. State of the art in the application of QSAR techniques for predicting mixture toxicity in environ-mental risk assessment. SAR QSAR Environ Res. 2015;26(1):41-59. doi: 10.1080/1062936X.2014.984627. PMID: 25608956.
75. Popa ML, Albulescu R, Neagu M, Hinescu ME, Tanase C. Multiplex assay for multiomics advances in person-alized-precision medicine. J Immunoassay Immunochem. 2019;40(1):3-25. doi: 10.1080/15321819.2018.1562940. Epub 2019 Jan 11. PMID: 30632882.
76. Calabrese EJ, Baldwin LA. Toxicology rethinks its central belief. Nature. 2003;421(6924):691-2
77. Birnbaum LS. Environmental chemicals: evaluating low-dose effects. Environ Health Perspect. 2012;120(4):A143-4. doi: 10.1289/ehp.1205179. PMID: 22470049; PMCID: PMC3339483.
78. Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE, Villeneuve DL. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem. 2010;29(3):730-41. doi: 10.1002/etc.34. PMID: 20821501.
79. OECD. 2013. Guidance document on developing and assessing adverse outcome pathways. Series on testing and assessment no. 184. Environment, Health and Safety Division, Environment Directorate. Paris, France: Organisation for Economic Co-operation and Development.
80. OECD. 2018. Considerations for assessing the risks of combined exposure to multiple chemicals. Series on test-ing and assessment no. 296. Environment, Health and Safety Division, Environment Directorate. Paris, France: Organisation for Economic Co-operation and Development.
81. Ashauer R, O'Connor I, Escher BI. Toxic Mixtures in Time-The Sequence Makes the Poison. Environ Sci Technol. 2017;51(5):3084-3092. doi: 10.1021/acs.est.6b06163. Epub 2017 Feb 17. PMID: 28177231.
82. Bopp SK, Kienzler A, Richarz AN, van der Linden SC, Paini A, Parissis N, Worth AP. Regulatory assessment and risk management of chemical mixtures: challenges and ways forward. Crit Rev Toxicol. 2019; 49(2):174-89
83. Kleinekorte J, Fleitmann L, Bachmann M, Kätelhön A, Barbosa-Póvoa A, von der Assen N, Bardow A. Life Cycle Assessment for the Design of Chemical Processes, Products, and Supply Chains. Annu Rev Chem Biomol Eng. 2020;11:203-233. doi: 10.1146/annurev-chembioeng-011520-075844. Epub 2020 Mar 27. PMID: 32216728.
84. SCHER, SCENIHR, SCCS. 2012. Toxicity and assessment of chemical mixtures. European Union. doi:10.2772/21444.
85. European Commission. 2012. Communication from the commission to the council - the combination effects of chemicals. Chemical mixtures. Brussels, 31.5.2012, COM(2012) 252 final.
86. Niedzwiecki MM, Walker DI, Vermeulen R, Chadeau-Hyam M, Jones DP, Miller GW. The Exposome: Molecules

to Populations. Annu Rev Pharmacol Toxicol. 2019;59:107-127. doi: 10.1146/annurev-pharmtox-010818-021315. Epub 2018 Aug 10. PMID: 30095351.
87. Andrianou XD, Makris KC. The framework of urban exposome: Application of the exposome concept in urban health studies. Sci Total Environ. 2018;636:963-967. doi: 10.1016/j.scitotenv.2018.04.329. Epub 2018 May 3. PMID: 29729514.
88. Escher BI, Hackermüller J, Polte T, Scholz S, Aigner A, Altenburger R, Böhme A, Bopp SK, Brack W, Busch W, Chadeau-Hyam M, Covaci A, Eisenträger A, Galligan JJ, Garcia-Reyero N, Hartung T, Hein M, Herberth G, Jahnke A, Kleinjans J, Klüver N, Krauss M, Lamoree M, Lehmann I, Luckenbach T, Miller GW, Müller A, Phil-lips DH, Reemtsma T, Rolle-Kampczyk U, Schüürmann G, Schwikowski B, Tan YM, Trump S, Walter-Rohde S, Wambaugh JF. From the exposome to mechanistic understanding of chemical-induced adverse effects. Environ Int. 2017;99:97-106. doi: 10.1016/j.envint.2016.11.029. Epub 2016 Dec 8. PMID: 27939949; PMCID: PMC6116522.
89. Lutz WK. Differences in individual susceptibility to toxic effects of chemicals determine the dose-response relationship and consequences of setting exposure standards. Toxicol Lett. 2002;126(3):155-8. Doi: 10.1016/s0378-4274(01)00458-1. PMID: 11814700.
90. Calabrese EJ. Origin of the linearity no threshold (LNT) dose-response concept. Arch Toxicol. 2013;87(9):1621-33. doi: 10.1007/s00204-013-1104-7. Epub 2013 Jul 26. PMID: 23887208.
91. Calabrese EJ. The threshold vs LNT showdown: Dose rate findings exposed flaws in the LNT model part 1. The Russell-Muller debate. Environ Res. 2017 Apr;154:435-451. doi: 10.1016/j.envres.2016.12.006. Epub 2017 Jan 18. PMID: 28109526.
92. Li L, Dingsheng L. Inter-Individual Variability and Non-linear Dose-Response Relationship in Assessing Hu-man Health Impact From Chemicals in LCA: Addressing Uncertainties in Exposure and Toxicological Suscep-tibility. Frontiers in Sustainability. 2021; 2: 17. Doi: 10.3389/frsus.2021.648138.
93. Calabrese EJ. The linear No-Threshold (LNT) dose response model: A comprehensive assessment of its histori-cal and scientific foundations. Chem Biol Interact. 2019 Mar 1;301:6-25. doi: 10.1016/j.cbi.2018.11.020. Epub 2019 Feb 11. PMID: 30763547.
94. 95 Calabrese EJ. Ethical failings: The problematic history of cancer risk assessment. Environ Res. 2021;193:110582. doi: 10.1016/j.envres.2020.110582. Epub 2020 Dec 5. PMID: 33290793.
95. Turin TC, Okamura T, Afzal AR, Rumana N, Watanabe M, Higashiyama A, Nakao Y, Nakai M, Takegami M, Nishimura K, Kokubo Y, Okayama A, Miyamoto Y. Hypertension and lifetime risk of stroke. J Hypertens. 2016;34(1):116-22. Doi: 10.1097/HJH.0000000000000753. PMID: 26556566.
96. Buonacera A, Stancanelli B, Malatino L. Stroke and Hypertension: An Appraisal from Pathophysiology to Clin-ical Practice. Curr Vasc Pharmacol. 2019;17(1):72-84. doi: 10.2174/1570161115666171116151051. PMID: 29149815.
97. Koman PD, Singla V, Lam J, Woodruff TJ. Population susceptibility: A vital consideration in chemical risk evaluation under the Lautenberg Toxic Substances Control Act. PLoS Biol. 2019;17(8):e3000372. doi: 10.1371/journal.pbio.3000372. PMID: 31465433; PMCID: PMC6715167.
98. Bollati V, Ferrari L, Leso V, Iavicoli I. Personalised Medicine: implication and perspectives in the field of occupational health. Med Lav. 2020;111(6):425-444. doi