Journal of Medicine and Health (ISSN 2155-7977)
June 2022, Volume 1, No. 1, pp. 1-5
Doi: 10.15341/jmh(2155-7977)/01.01.2022/001
Academic Star Publishing Company, 2022
www.academicstar.us

Risk Assessment of Chemical Mixtures

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Abstract: Humans are exposed daily to numerous mixtures of chemicals present in their environment instead of just a single chemical. The classical health risk assessment for regulatory decisions uses the chemical toxicity of a single test agent one at a time. Comparison of the toxicity of even a simple chemical mixture with that of its parts often shows significant differences. Therefore, the classical chemical risk assessment paradigm does not necessarily represent real-world human exposure, making a risk assessment of chemical mixtures an even more complex process. *In vitro*, *in silico*, organs-on-a-chip, and 3D cell culture models are examples of alternative approaches that have been used for toxicity screening. Recent advances in new "omics" technologies continue to provide useful data for hazard and risk assessment of chemical mixtures. The new developing genomic and epigenomic technologies also show promise for human health risk assessment. However, all these exciting experimental models and tools must be validated and accepted before they can be used to support risk assessments of chemical mixtures for regulatory approval.

Key words: mixtures, chemical mixtures, health risk assessments, NAMs

1. Introduction

Safety assessment is required for the regulation of industrial and household chemicals, food additives, pesticides, and drugs, many of which are complex mixtures. Such a health assessment process consists of four steps: (a) hazard identification, (b) dose-response relationship, (c) exposure assessment, and (d) risk characterization (U.S. National Academy of Sciences, 1981) typically undertaken using *in vivo* animal studies for hazard identification and dose-response assessment.

Guidelines for the health risk assessment of chemical mixtures were published by U.S. Environmental Protection Agency (EPA) in 1986 [1]. It allowed the risk assessment of chemical mixtures to be determined by the toxic or carcinogenic properties of the components in the mixture. This dose additive model predicted reasonably well the toxicities of mixtures composed of a substantial variety of both similar and dissimilar compounds [2]. In addition, in 2014, the Joint Research Centre of The European Union issued its 136-page report on the assessment of mixtures — Review of Regulatory Requirements and Guidance[1].

Following shortly, the Organization for Economic Co-Operation and Development (OECD) released Considerations for Assessing the Risks of Combined Exposure to Multiple Chemicals (No. 296)[2]. The goal of this document was to overview the technical aspects of the various approaches and methodologies available for the assessment of risks from combined exposures to multiple chemicals.

The toxicity of mixtures is a problem that needs investigation. However, when assessing the toxicity of chemical mixtures, it is important to test the null hypothesis of no interactions. Only upon its rejection

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[1] Available online at: https://publications.jrc.ec.europa.eu/repository/handle/jrc90601.

[2] Available online at: https://www.oecd.org/chemicalsafety/risk-assessment/considerations-for-assessing-the-risks-of-combined-exposure-to-multiple-chemicals.pdf.

should the possibility of synergistic interactions be considered.

In classical health risk assessments, the dose-response relationships in test animals have been determined using high doses of the test material administered frequently by a different route of exposure compared to the real-world human exposures. Humans are generally exposed by not only the oral route but also by dermal contact and inhalation. Extrapolations of experimental animal results from animal to human, from high dose to low dose, from the experimental route of exposure to real-world exposure are major challenges. Also, animal studies are costly and time-consuming.

To reduce the cost and time of classical animal studies, the U.S. National Academy of Sciences published its landmark report *Toxicity Testing in the 21st Century: A Vision and a Strategy* in 2007 [3]. This report suggested *in vitro* studies in human cells as alternatives to classical animal studies to determine the dose of the test material required for the health hazard assessment for regulatory decisions. However, Tice and his colleagues (2013) [4] cautioned in 2013 that the task of converting completely to such approaches has several difficulties including 1) "perfect" assays do not exist; 2) coverage of all chemicals of interest is incomplete (i.e., volatiles), 3) a high throughput system for measuring the free concentration of a compound *in vitro* is not yet available; 4) the lack of xenobiotic metabolism in virtually all *in vitro* assays, interactions between cells are poorly captured; 5) distinguishing between statistical and biological significance is difficult; 6) extrapolating from *in vitro* concentration to *in vivo* dose or blood levels is not straightforward; 7) assessing the effects of chronic exposure conditions *in vitro* is not possible; 8) identifying when a perturbation to a gene/pathway would lead to an adverse effect in animals or humans remains a challenge, and; 9) achieving routine regulatory acceptance of the developed prediction models is years away. Several of their cautions still exist in 2021. In addition, the use of uncertainty factors for single chemical exposure by a single route, much less mixtures via multiple routes, remains a regulatory concern particularly in light of new approach methodologies (NAMs) which are challenging the traditional "norm" of current regulatory risk assessments [5].

The classical health risk assessment typically relies on developing the toxicity profile for each chemical even in the case of simple mixtures of chemicals much less complex mixtures of chemicals such as mixtures found in superfund sites or air pollution or botanical supplements. However, humans are rarely if ever exposed to a single chemical but are exposed to mixtures of chemicals present in their environment, often daily and to different mixtures, often by multiple routes. Therefore, the classical chemical risk assessment, based on individual chemicals, does not represent the real-world human exposure to mixtures of chemicals occurring at the same or different times [6]. Furthermore, it has been demonstrated that the combination of toxicities of individual components of a chemical mixture is not always additive and can result in variations of toxicity depending on the interactions of the individual component of the chemical mixture and routes of exposure [7]. Therefore, a framework for the hazard assessment for chemical mixtures is needed. An assessment of chemical mixtures should represent all the available integrated scientific evidence on their potential individual toxicities [8] as well as the combined toxicity of the mixture.

The toxicity of a chemical mixture, therefore, may not always be additive of the toxicity of the individual components of the mixture but may elicit synergistic toxicity. Hayes et al. (2006) [9], for example, studied the effects of nine pesticides individually and in combination on the time to foreleg emergence and complete tail resorption in *Rana pipiens* and concluded that the mixture had a greater than additive effect than that of the individual chemicals in the mixture.

2. Risk Assessment of Botanicals

Various medicinal plants have been used in health care since ancient times. Plants are a promising but complex source of drugs and dietary supplements. Risk assessment of botanicals is challenging because of their chemical complexity. In addition, plant products are prepared differently from different plants and different plant parts. Identification of the active ingredient is often a major issue. Extracts of natural products consist of a mixture of different individual components, Determining the safety of individual components can lead to the evaluation of the safety of the natural product extract. Booth et al. (2012) [10] used an aqueous extract of cranberry *(Vaccinium macrocarpon* Aiton) leaves to prove this hypothesis. Clemens et al (2017) [11] have discussed the uncertainty of hazard identification and risk assessments of palm oil and threats to a critically important food source. Constable et al. (2017) [12] have presented an integrated approach to the safety assessment of food additives. Hayes et al. (2019) [13] have discussed various approaches to risk assessment of complex chemical mixtures using new emerging technologies. An international roundtable meeting brought together scientists to discuss the needs, available tools, and ongoing data gaps in the botanical safety risk assessment process [14]. The identified critical areas and data gaps included better context on the history of use, systematic assessment of the weight of evidence, use of *in silico* approaches, the inclusion of threshold of toxicological concern considerations, individual substances/matrix interactions of plant constituents, assessing botanical-drug interactions and adaptations needed to apply to *in vitro* and *in vivo* pharmacokinetic modelling of botanical constituents.

2.1 Alternate Animal Models for Risk Assessment of Chemical Mixtures

High cost and time-consuming evaluation of animal testing for human health risk assessment have propelled the use of alternative animal models and emerging new technologies for risk assessment. Zebrafish and the worm, *Caenorhabditis elegans,* are just two examples. Zebrafish have been a useful alternate animal model for toxicity testing [15]. Zebrafish embryos have been used as an alternate animal model for risk assessment of chemical mixtures [16]. *Caenorhabditis elegans* is another alternate model that is being used for the health risk assessment of chemical mixtures [17].

2.2 In Vitro and in Silico Models for Health Risk Assessment of Chemical Mixtures

The OECD (2018) [18] allows *in vitro* data to be used for risk assessment of chemical mixtures. New *in vitro*, *in silico*, organs-on-a-chip, and 3D cell culture models are being developed as predictive toxicity screening [5, 19]. Quantitative modelling that uses systems toxicology approaches can identify exposure-induced cellular and molecular alterations that would not be detected by standard toxicology assays [13]. All these tools, however, must be validated and accepted before they can be used for the risk assessment of chemical mixtures [20, 21].

2.3 Estimation of Combined Toxicities of Chemical Mixtures

A risk probability-based method for evaluation of combined health risks of a chemical mixture of aflatoxin B1 and microcystin LR was developed by Li et al. (2020) [22]. This approach may be useful for estimating the combined effects of chemical mixtures for human health by dietary exposure. Other model analyses indicate that the observed synergistic effects are due to response addition or response multiplication joint actions and that most synergistic joint actions are non-interactive and are governed by the dose-response relationships of individual toxicants [23]. Cheng and colleagues have proposed an alternative classification strategy to integrate chemical and toxicological data including combination effects of chemical mixtures in influencing toxicological responses [24].

The Online Chemical Modelling Environment (OCHEM)[3] is a web-based platform that provides tools for the automation of typical steps necessary to create a predictive QSAR/QSPR model. Until recently, the OCHEM was limited to the processing of individual compounds; however, recently the OCHEM has been extended with a new ability to store and model properties of binary non-additive mixtures. Liess and colleagues have provided an additional approach for the evaluation of the combined toxicant effect as R package and as Indicate model [25][4].

The toxicity of a simple chemical mixture with that of its components often shows significant differences. Therefore, the classical chemical risk assessment paradigm does not necessarily represent real-world human exposure, making assessing chemical mixtures an even more complex process. *In vitro*, *in silico*, organs-on-a-chip, and 3D cell culture models are examples of alternative approaches that are being used for toxicity screening. Recent advances in new "omics" technologies continue to provide useful data for hazard assessment of chemical mixtures. Genomic and epigenomic technologies have also shown promise for human health risk assessment. However, all these exciting experimental models and tools must be validated and accepted before they can be used to support risk assessments of chemical mixtures for regulatory approval.

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