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Letter

Cardiotoxicity of Chemicals: Current Regulatory Guidelines, Knowledge Gaps, and Needs

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In a recently published article, Daley et al. (2022) emphasized the need for a new approach to assess cardiotoxicity of environmental compounds, an issue that has been neglected to date. They described how efforts for the evaluation of cardiotoxicity have so far focused on pharmaceuticals and the necessity of new approach methodologies (NAMs) that do not directly rely on animals beyond the pharmaco-regulatory sector.
Here, we aim to add to that paper by providing information on the specific limitations of the current regulations for a) chemicals, biocides, and pesticides substances, b) human variability including the elderly population, and c) chemical mixtures. We also briefly indicate how each of these specific limitations could be overcome using NAMs.

Limitations of regulatory test guidelines for cardiotoxicity assessment of chemicals, biocides, and pesticides

As described in Daley et al. (2022), "*cardiovascular diseases (CVD) [are] the leading cause of mortality worldwide …* [and] *an estimated 7-23% of CVD can be attributed to environmental factors such as air pollution, occupational hazards, and agricultural run-off … [but] … there is a shortage of knowledge regarding the cardiac-specific risks presented by environmental toxicants*". Methods to specifically evaluate effects on the cardiovascular system are currently described in the ICH guidelines for pharmaceuticals. However, the available methods are too limited for a sufficient evaluation of pharmaceuticals and environmental contaminants (Daley et al., 2022). The *in vivo* guideline studies are in conflict with the 3R goals, are economically and time resource intensive, and are generally subject to uncertainties in extrapolation between different animal species and humans. The existing *in vitro* guideline studies (hERG channel tests) do not adequately cover all relevant mechanisms. Novel human-relevant NAMs are based, among others, on human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM), in 2D or 3D cultures, and focus not only on electrophysiological but also structural and/or contractility endpoints, as discussed by Daley et al. (2022). They may provide a more accurate, species-specific assessment for the clinical risk of proarrhythmia and structural and contractile toxicity.

Adding to the observations of Daley et al. (2022), we note that cardiotoxicity has been vastly neglected in the evaluation of chemicals, pesticides, and biocides. Currently, their production and use is regulated by the REACH regulation (EC) No 1907/2006 (EU, 2006), the plant protection products regulation (EC) No 1107/2009 (EU, 2009), and the biocidal products regulation (EU) No 528/2012 (EU, 2012), respectively. The regulation of pesticides and biocides requires toxicological data from a number of OECD Test Guidelines (TGs), including information on potential toxicity to specific target organs. Similar requirements are applied under REACH for chemicals that are produced in large quantities. More specifically, toxicological data must be collected from repeated toxicity studies (28-day, 90-day or up to 2 years and extended one-generation studies). However, the only cardiac-specific endpoints included are cardiac weight, necropsy, and histopathology. The potential for malformations of the heart is analysed in prenatal developmental studies (OECD, 2022). To our knowledge, the need for more specific cardiotoxicity assessments has not yet been recognized, which may be due to the lack of available cardiotoxicity-specific data, i.e., a catch-22 situation. In the future, high throughput NAMs may be used to derive a hypothesis for any specific mode of action (MoA) and target organ. This broad assessment may be followed up with more complex NAMs as needed, depending on considerations of exposure and acceptable uncertainty, which may vary between different regulatory purposes (OECD, 2017).

Moreover, currently, high doses are administered in guideline animal studies in order to increase the chance of achieving statistically significant effect sizes to derive limit values on the one hand, and sufficient severity to allow classification under current regulations on the other. As a consequence, multiple and unspecific effects may be induced, leaving the specific MoAs and the more subtle effects that may lead to toxicity in the longer term, including those resulting in heart failure (HF), undetected. In the future, NAMs may be used to test for early biomarkers of disease at low concentrations well below those that cause generic cytotoxicity. Such biomarkers may be validated using existing basic mechanistic knowledge and available human data, structured according to the concept of cardiovascular failure modes (Krishna et al., 2021) and/or more detailed adverse outcome pathways (AOPs). Further work at the science-regulatory interface will be necessary to evolve the recognition that early biomarkers of disease occurring at low concentrations may be scientifically adequate and useful for

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derivation of limit values and for classification. This recognition may mature with the increasing understanding that an effect should be considered as adverse if it reduces the capacity of an organism or population to compensate for the multitude of additional stressors in the real world. The latter cannot be tested in any animal study (Paparella et al., 2017, 2020).

Finally, we note that data variability within and between animal experiments is not informative for potential variability within human (sub)populations. Therefore, NAMs have the important advantage that they can be more easily optimized for low variability, high statistical power, and appropriate data acceptance criteria, *inter alia* taking into account historical negative and positive control data. Potential human variability may be modelled based on available human data for any set of reference chemicals or effect types. In addition, human-relevant NAMs may be used to inform on any potential chemical-specific or effect type-specific human variability, as briefly indicated in the following paragraphs.

Cardiotoxic risk for the elderly population

As the elderly population, who have a higher incidence of HF[2], is increasing (22% of global population will be older than 60 years by 2050[3], the need for approaches to assess the cardiotoxic potential of chemical stressors in the elderly population is rising. Yet, current approaches do not specifically address the susceptibility of this population.

In animal studies, later life stages beyond the first appearance of senescence biomarkers (i.e., about two thirds of the rodent life span) are generally not tested because this may increase variability, which negatively affects the power of statistical assessment. In addition, life expectancy and timing of life stages (neonate, juvenile, adult, senescence biomarkers) vary among species (Paparella et al., 2017), making life-stage specific animal test results of uncertain relevance to humans. Furthermore, the outcome of a dose-response assessment in animal studies, such as the dose at which a biological response is first observed (point of departure, PoD), needs to be extrapolated to an equipotent human dose, accounting for species differences in kinetics and target organs/MoAs (i.e., toxicodynamics). The standard deterministic approach, which applies default assessment factors (usually 100) for dose adjustment, results in limit values without confidence intervals and without the option to derive data-based limit values that are specific to a human subpopulation.

Alternatively, based on work by the WHO, a probabilistic hazard characterization can provide a human dose including a confidence interval that is protective for a specific fraction, e.g., 95% or 99% of the population (WHO, 2018). This probabilistic approach is based on an assessment of a probability distribution for PoDs from animal studies as well as assessment factor distributions derived from inter-species and human intra-species variability data, which were available for about 100 chemicals for each aspect. However, the information on human variability used in the WHO (2018) probabilistic approach is largely derived from available data from healthy volunteers for clinical trials, thus lacking information on the susceptible elderly population nonetheless. Additionally, if the limit value is aimed at a high level of protection where no more than 1 in 10^6 people are exposed to risk, the uncertainty (confidence interval) for the limit value cannot be quantified because of data limitations at the extreme ends of the assessment factor distributions for human variability.

In summary, current regulatory risk assessments for chemicals, pesticides, and biocides cannot provide information on specific risks and uncertainties for the aging population, neither based on animal test data nor on deterministic or probabilistic accounting for human variability. The situation is similar for the regulation of pharmaceuticals, although comparatively more data are collected during pharmacovigilance.

As indicated by Daley et al. (2022), information on potential human variability could be obtained from *in vitro* hiPSC-CM-based models (Burnett et al., 2021). Yet, further work is needed to integrate specific risk factors of the elderly population, like aged connective tissue, which could be applied in more sophisticated microphysiological 3D systems. Such information could be used to modify the generic overall human variability assessment factors. Alternatively, warning labels could be issued for chemicals that are specifically hazardous to the elderly population, e.g., if NAM-derived PoDs for aged models are more than 10-fold below those for the standard "young" models.

Mixture assessment for cardiotoxicity

Humans are usually exposed to multiple chemical stressors simultaneously, thus cardiotoxicity may be the result of the environmental exposure to a mixture of chemicals. However, practical tools for a scientifically informed toxicological assessment of mixtures remain limited.

In the EU, the toxicity of products containing mixtures of ingredients is assessed either under authorization procedures (pesticides, biocides) or under the responsibility of the marketer (chemical products). The ingredients present in these mixtures may have independent, additive, synergistic or antagonistic effects. Chemical mixtures are usually not tested, but the toxicity of the mixture is estimated based on data for its constituents. In the absence of test data for the mixture in products that are regulated by risk assessment, the current standard approach to mixture risk assessment is to assume additive effects of the ingredients within a mixture, unless there is evidence to the contrary. In cases where the risk assessment concludes an unacceptable risk (exposure to limit value ratio >1), a more detailed MoA analysis is performed to evaluate whether some of the ingredients have independent effects (ECHA, 2017). However, such MoA analyses are often limited by the availability of suitable data. Consequently, for data-poor situations, even more pragmatic solutions are in discussion, which engage mixture allocation factors (MAFs) depending on the number of mixture components (Kemikalieinspektionen, 2021).

The reasons for such pragmatic approaches to mixture assessment are that the high number of mixtures available on the market and the large variability of potentially contaminated environmental media (water, air, soil, food) make practical testing difficult. Moreover, the current regulatory dependence on animal testing is significantly adding to the challenges of mixture assessment due to their high resource requirements in the form of animals, time and costs.

[2] NIH – National Institutes of Health (2018). *Heart Health and Aging, National Institute on Aging*. Retrieved Nov 9, 2022 from https://www.nia.nih.gov/health/heart-health-and-aging

[3] WHO fact sheet of October 1, 2022, retrieved Nov 9, 2022. https://www.who.int/news-room/fact-sheets/detail/ageing-and-health

Individual assessment of compounds using NAMs could provide additional data for human MoA assessment and give information on independent, additive, synergistic, or antagonistic effects in mixtures, thus, refining the classification or risk assessment of mixtures. Alternatively, entire mixtures may be tested as such using NAMs within Integrated Approaches to Testing and Assessment (IATAs) provided that the applicability domain of the NAMs does not exclude mixtures on the basis of clear scientific evidence. Potential improvements in the regulation of mixture effects have been studied for many years (Bopp et al., 2019). The results of ongoing projects (PANORAMIX[4]) may provide further insights and tools for improved chemical regulation of mixtures.

In conclusion, current regulatory guidelines inadequately cover the assessment of cardiotoxicity for pharmaceuticals, but even more poorly for chemicals, biocides, and pesticides to which humans may be exposed in the workplace or through food and the environment (Figure 1). Available tests for the assessment of the few required cardiotoxicity-related endpoints rely heavily on animal studies and suffer from difficulties in identifying MoAs, interspecies differences, high costs, and low throughput. As far as *in vitro* methods are recommended (for pharmaceuticals), they do not include information on heart structure and contractility changes and have limited predictability for the potential of chemicals to induce very dangerous torsade de pointes. Especially for environmental exposures to pharmaceuticals, chemicals, biocides, and pesticides that are likely to be lifelong and multi-route, the NAMs that are being developed should be able to address the distinct toxicological profiles of small chronic doses in complex mixtures and in susceptible populations such as the elderly.

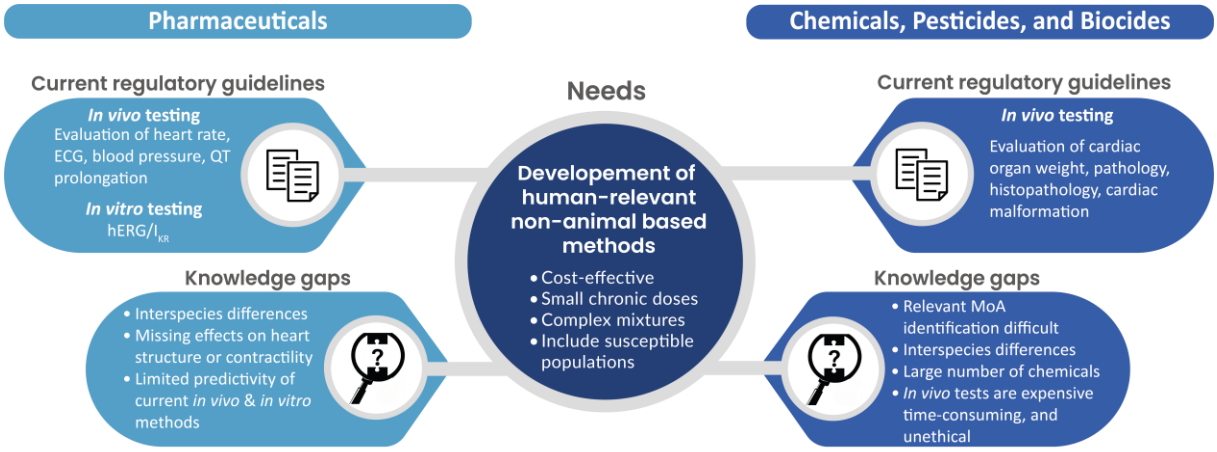

Figure 1: Current regulatory guidelines, knowledge gaps, and needs in cardiotoxicity evaluation

Outlook

There are ongoing efforts, such as the initiation of the EU 2020 Horizon project ALTERNATIVE[5] (2021 to 2024) to address the aforementioned challenges. The project aims to develop a novel platform that will enable regulators and industry to identify, quantify, and prevent cardiotoxic co-exposures to industrial chemicals and pharmaceuticals in a cost-effective way. Within this project, a 3D physiological cardiac tissue model composed of hiPSC-CMs and human coronary artery cells is being developed. Two versions are being created, one representing a healthy young heart and the other an aged heart. The latter is achieved by developing a more rigid extracellular matrix scaffold. The 3D model will be combined with high-throughput omics and computational modelling to identify biomarkers of cardiotoxicity and derive PoDs using kinetic quantitative *in vitro* to *in vivo* (QIVIVE) modelling. Selected chemicals will be tested separate and in mixture with the purpose to demonstrate the applicability of the established approach for the identification of human-relevant cardiotoxic mechanisms, PoDs, and mixture effects.

Systematic reviews will be conducted to assess epidemiological and toxicological evidence for cardiotoxicity of chemicals. The evidence from the systematic reviews will be integrated into novel AOPs for cardiotoxicity to support the drafting of an integrated approach to testing and assessment (IATA). This IATA shall facilitate a tiered approach to cardiotoxicity assessment according to available OECD concepts for risk assessment based on exposure information and NAM data (OECD, 2017), including a broad set of available methods beyond those being developed within the ALTERNATIVE project. Ultimately this IATA shall provide a more robust basis for regulatory decision-making. Regular newsletters are informing on the progress of the ALTERNATIVE project.

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Conflict of interest

The authors declare no conflicts of interest.