WHITE PAPER ON REGULATION OF CHEMICAL EXPOSURES AND CARDIAC HEALTH: CURRENT GAPS AND FUTURE SOLUTIONS

INTRODUCTION: THE CARDIOTOXIC FACTORS AFFECTING OUR HEARTS

Heart diseases are a global health challenge. Worldwide, they account for nearly 18 million deaths per year[1] and an estimated 3.9 million deaths in Europe[2]. While behaviour and preexisting health conditions are major risk factors[3], there's another silent threat: omnipresent air pollution[4] and a vast array of lesser-known culprits, chemicals, ranging from those in drugs and pesticides to metals and natural compounds[5].

Air pollution has been identified as a significant player in heart disease[6]. Pollutants like particulate matter, nitrogen dioxide, and heavy metals have been shown to increase the likelihood of heart failure[7]. Additionally, with modern society's heavy reliance on countless chemical compounds, we are continually exposed to a

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[1] *Cardiovascular diseases (CVDs)*. Jun. 20, 2023]; Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

[2] Network, E.H. *European Cardiovascular Disease Statistics*. 2017 Jun. 20, 2023]; Available from: www.ehnheart.org

[3] Munzel, T., et al., *Environmental risk factors and cardiovascular diseases: a comprehensive expert review.* Cardiovasc Res, 2022. **118**(14): p. 2880-2902.

[4] Bhatnagar, A., *Environmental Determinants of Cardiovascular Disease.* Circ Res, 2017. **121**(2): p. 162-180.

[5] Klaassen, C.D., *Casarett & Doull's Toxicology: The Basic Science of Poisons, 9th Edition*. 2018: McGraw-Hill Education.

[6] Shah, A.S., et al., *Global association of air pollution and heart failure: a systematic review and meta-analysis.* Lancet, 2013. **382**(9897): p. 1039-48.

[7] Atkinson, R.W., et al., *Long-term exposure to outdoor air pollution and incidence of cardiovascular diseases.* Epidemiology, 2013. **24**(1): p. 44-53.

spectrum of potential cardiac threats. Notably, some studies suggest that pesticides used in agriculture might escalate the risk of heart failure[8].

The complex interplay of risk factors (Figure 1), from our exposure to toxins and pollution to our age and behavior, means that heart disease can manifest differently in everyone[9]. And it's not just about the heart; many harmful compounds affect multiple organs through various pathways – making it challenging to trace back and identify the direct cardiac consequences of specific chemicals.

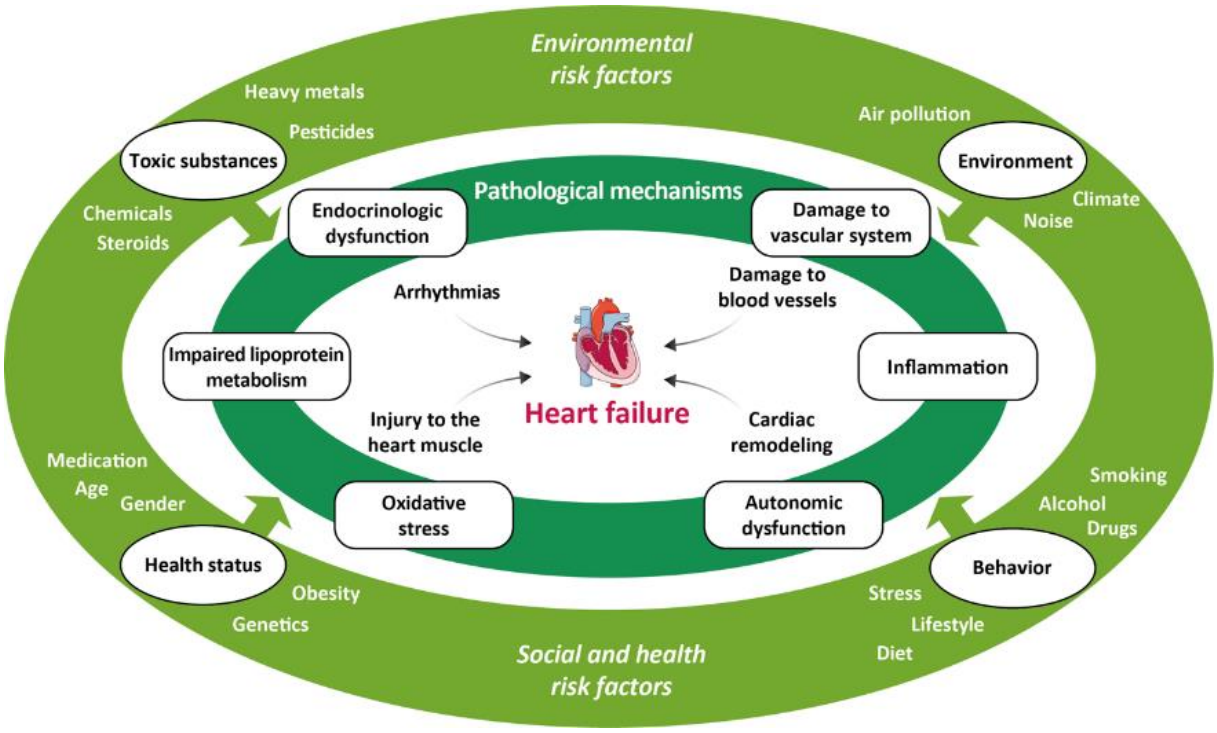

Figure 1. Social, health, and environmental risk factors to heart failure (modified from (9).

[8] Zago, A.M., et al., *Pesticide exposure and risk of cardiovascular disease: A systematic review.* Glob Public Health, 2022. **17**(12): p. 3944-3966.

[9] Narita, K. and E. Amiya, *Social and environmental risks as contributors to the clinical course of heart failure.* Heart Fail Rev, 2021. **27**(4): p. 1001-1016.

ARE CURRENT GUIDELINES SUFFICIENTLY PROTECTIVE OF CARDIOTOXICITY?

Pharmaceuticals:

The International Council for Harmonisation (ICH)[10] [11] [12] [13] [14]offers directives that orchestrate the drug development continuum, ensuring the therapeutic agents under review are commensurate with human safety parameters. Yet, the extant methodologies elicit several notable reservations:

- **Animal Model Inter-Species Differences:** Animals, while biologically similar to humans in many ways, have significant physiological, metabolic, and genetic differences. This can lead to incorrect assumptions when trying to translate animal data to human outcomes[15] [16]. What's safe for a rodent might not be safe for a human and vice versa.
- **Animal Welfare:** There's a growing concern worldwide about the ethics of using animals for testing. Exposing animals to potentially harmful chemicals or pharmaceuticals raises moral questions about causing them pain, distress, or even death.
- **Resource-intensive:** Animal studies, especially long-term ones, are costly. They require not only the animals but also the infrastructure to house and care for them, trained personnel, and time.
- **Challenges with Chronic Exposure:** Many chemicals and pharmaceuticals need long-term testing to understand potential chronic effects. With animal lifespans varying and sometimes being much shorter than human lifespans, these tests can miss long-term effects or become expensive when carried out for a prolonged time.
- **In vitro Assay Efficacy:** Recent advancements using human-derived cells, while promising, are yet to become mainstream in pharmaceutical safety assessment. Existing assays investigate the potential for a substance to cause arrhythmias by inhibiting a specific type of cardiac potassium channel, like the predominantly used hERG/IKR in vitro assay. Although sensitive, the assay currently doesn't comprehensively predict the full spectrum of how a drug might affect heart rhythms[17].

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[10] ICH. *SAFETY PHARMACOLOGY STUDIES FOR HUMAN PHARMACEUTICALS S7A*. 2000 01.09.2022]; Available from: https://database.ich.org/sites/default/files/S7A_Guideline.pdf.

[11]ICH. *THE NON-CLINICAL EVALUATION OF THE POTENTIAL FOR DELAYED VENTRICULAR REPOLARIZATION (QT INTERVAL PROLONGATION) BY HUMAN PHARMACEUTICALS S7B*. 2005 01.09.2022]; Available from: https://database.ich.org/sites/default/files/S7B_Guideline.pdf.

[12]Group, I.E.S.B.I.W., *ICH S7B Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential - Questions and Answers.* 2022.

[13]ICH. *GUIDANCE ON NONCLINICAL SAFETY STUDIES FOR THE CONDUCT OF HUMAN CLINICAL TRIALS AND MARKETING AUTHORIZATION FOR PHARMACEUTICALS M3(R2)*. 2009 01.09.2022]; Available from: https://database.ich.org/sites/default/files/M3_R2__Guideline.pdf.

[14]Harmonisation, I.C.f., *M3(R2) Implementation Working Group M3(R2) Guideline: Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals Questions & Answers (R2).* 2012.

[15] Daley, M.C., et al., *Beyond pharmaceuticals: Fit-for-purpose new approach methodologies for environmental cardiotoxicity testing.* ALTEX, 2022.

[16]Gintant, G., P.T. Sager, and N. Stockbridge, *Evolution of strategies to improve preclinical cardiac safety testing.* Nat Rev Drug Discov, 2016. **15**(7): p. 457-71.

[17]Colatsky, T., et al., *The Comprehensive in Vitro Proarrhythmia Assay (CiPA) initiative - Update on progress.* J Pharmacol Toxicol Methods, 2016. **81**: p. 15-20.

INDUSTRIAL CHEMICALS, PESTICIDES, AND BIOCIDES:

The Regulations on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)[18], Plant Protection Products (PPP)[19], and Biocidal Products (BPR)[20] represent the high standards set for the development and use of chemicals, pesticides, and biocides. Yet, these guidelines have their shortcomings:

- **Animal Model Limitations:** Similar to pharmaceuticals, testing largely relies on animal experiments with the same issues regarding limited extrapolation to humans. In addition, particularly the practice of using high doses to detect adverse effects in animals can mask nuanced mechanisms of action, including those potentially leading to heart failure in humans.
- **Volume of Chemicals vs. Testing Depth:** Thousands of new chemicals are introduced into the market annually in addition to a vast number of already existing chemicals. However, detailed toxicological evaluations have only been done for a fraction of them, leaving potential risks unidentified. Testing each of these chemicals on animals would require an enormous number of subjects.
- **Combinatorial Complexity:** Many chemicals may be safe on their own but can become harmful when combined with others. Testing for a broader set of relevant combinations is logistically unfeasible with animal models.

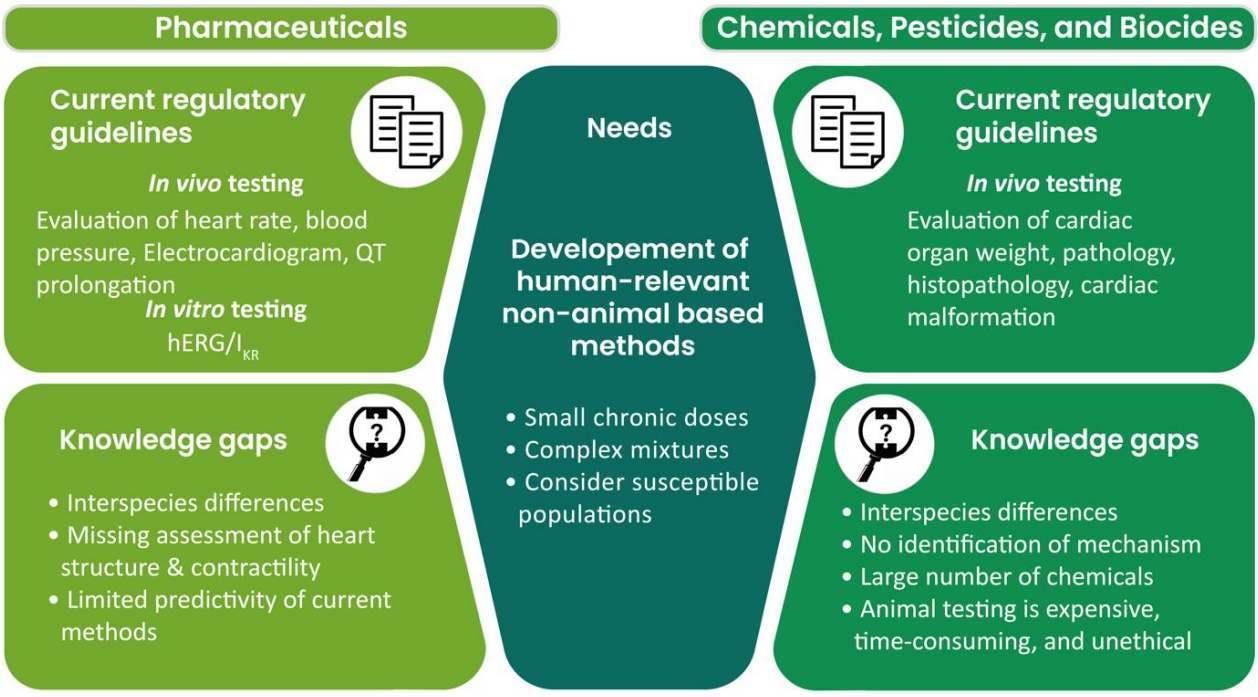

Figure 2. Current regulatory guidelines, knowledge gaps, and needs in cardiotoxicity evaluation. In vivo = animal testing; In vitro = non-animal-based testing

[18]EU. *REGULATION (EC) No 1907/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 December 2006*. 2006 Sept. 1, 2022]; Available from: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:136:0003:0280:en:PDF.

[19]EU. *REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009*. 2009 Sept. 1, 2022]; Available from: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0001:0050:EN:PDF.

[20]EU. *REGULATION (EU) No 528/2012 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 May 2012*. 2012 Sept. 1, 2022]; Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012R0528.

In essence, while we have structures in place to gauge the cardiotoxic risks of pharmaceuticals and chemical substances, there's a clear need for ongoing development to ensure thorough protection of human health. Embracing more human-centric evaluations and considering the unique challenges posed by environmental exposures can lead to a more accurate and comprehensive assessment (Figure 2)[21].

THE AGING POLULATION IS MORE SUSCEPTICLE TO CARDIOTOXIC RISKS

Modern societies are experiencing a rise in their aged populations, with global projections indicating about 22% being over 60 years old by 2050[22]. Heart failure is more prevalent among the elderly[23], making them especially vulnerable to cardiotoxic chemicals and drugs. Nevertheless, existing methods to evaluate toxicological reactions to chemicals rarely factor in this aging demographic.

Most regulatory guidelines rely on animal-derived data. A significant challenge here is that these animal studies rarely focus on the latter stages of life, leading to data that might not be truly reflective of aging human populations. Additionally, the lifespan disparities across species introduce another layer of uncertainty when applying animal-based findings to humans[24].

Current strategies that extrapolate animal data to human thresholds primarily use default assessment factors to compensate for species differences and human variability. While this approach does provide some boundary values, it fails to offer a clear picture of uncertainties, especially for specific population segments.

Presently, the regulatory landscape doesn't furnish detailed risk insights for the aged. Some fields, like human medicine, do have more data, particularly in the post-authorization phase. Incorporating comprehensive human variability data, especially concerning the elderly, could significantly enhance chemical risk assessments and lead to more targeted advisories for substances particularly harmful to the older population. In addition, human epidemiological studies have traditionally focused on clinical effects of chemical exposure, such as disease or death. However, emerging evidence suggests that preclinical organ effects, such as changes in organ structure or function, may also be important early warning signs of damage, especially in vulnerable populations such as the elderly. More research on preclinical organ effects from lifelong chemical exposure in the elderly is needed to identify people at risk of developing **cardiovascular** health problems.

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[21]Schaffert, A., et al., *Cardiotoxicity of chemicals: Current regulatory guidelines, knowledge gaps, and needs.* ALTEX, 2023. **40**(2): p. 337-340.

[22](WHO), W.H.O. *Ageing and health*. 2022 Jun. 20, 2023]; Available from: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health.

[23]Aging, U.S.N.I.o. *Heart Health and Aging | National Institute on Aging*. Jun. 20, 2023]; Available from: https://www.nia.nih.gov/health/heart-health-and-aging.

[24]Paparella, M., A. Colacci, and M.N. Jacobs, *Uncertainties of testing methods: What do we (want to) know about carcinogenicity?* ALTEX, 2017. **34**(2): p. 235-252.

CHEMICALS AND THEIR PATHWAYS TO HEART FAILURE: A BETTER WAY TO CARDIOTOXICITY ASSESSMENT

Traditional toxicity testing has predominantly been based on observing adverse outcomes in animals after exposure to chemicals[25]. This approach, however, often doesn't elucidate the specific mechanisms that lead to these outcomes. Picture it as trying to understand the inner workings of a black box without ever peeking inside. Not only is this approach somewhat limited in its scope, but it's also highly dependent on animal testing – with all its associated challenges, as we've explored in earlier chapters.

Delving into the specifics, let's consider the heart. At the forefront of its operation are the cardiomyocytes, cells that constitute a significant portion of the heart's mass. Depending on the chemical stressor, different molecular reactions can be initiated. For instance, some chemicals might produce reactive oxygen species leading to oxidative stress, while others might interfere with mitochondrial complexes or inhibit ion channels, both vital for cardiac contractility [26] [27](Figure 3). Such molecular disruptions can place the cells under stress, and in extreme situations, lead to cell death (Figure 3)[28]. There's also the potential disruption of ion balance within the heart cells. At the tissue level, these events could result in decreased heart contractility and changes like cardiac remodelling manifested as an abnormal increase in cell size and fibrosis (scarring) [5]. This can also give rise to rhythm irregularities, or arrhythmias [5]. Expanding the view to the organ as a whole, adverse effects can include ventricular dysfunction, elongation of the QT interval on ECG readings, and the development of a life-threatening type of arrhythmia known as "torsades de pointes" (Figure 3) [5]. Continuous exposure and unchecked reactions might progress to heart failure and, in some cases, sudden cardiac deaths.

In summary, the heart's response to chemical exposures underscores the importance of understanding toxicity at a granular level. Given the pivotal role of cardiomyocytes and their vulnerability, it's essential to study and mitigate potential chemical threats for better heart health.

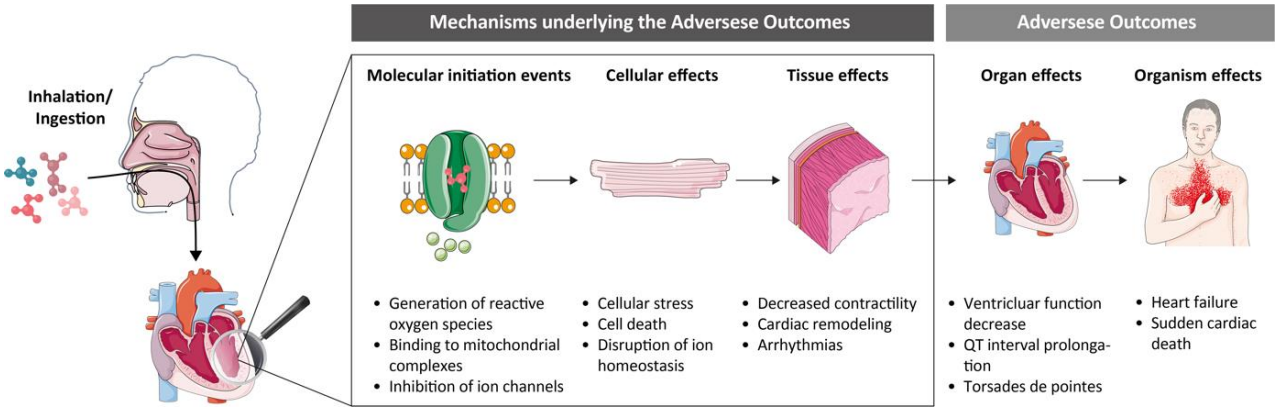

Figure 3. From Chemical Exposure to Adverse Heart Outcomes.

[25]Krewski, D., et al., *Toxicity testing in the 21st century: a vision and a strategy.* J Toxicol Environ Health B Crit Rev, 2010. **13**(2-4): p. 51-138.

[26]Cosselman, K.E., A. Navas-Acien, and J.D. Kaufman, *Environmental factors in cardiovascular disease.* Nat Rev Cardiol, 2015. **12**(11): p. 627-42.

[27]Werbner, B., et al., *The dynamic interplay between cardiac mitochondrial health and myocardial structural remodeling in metabolic heart disease, aging, and heart failure.* J Cardiovasc Aging, 2023. **3**(1)

[28]Piquereau, J., et al., *Mitochondrial dynamics in the adult cardiomyocytes: which roles for a highly specialized cell?* Front Physiol, 2013. **4**: p. 102.

EUROPEAN POLICY: A STRATEGIC RESPONSE

The European Union aims for a future free from harmful chemicals[29]. A key element of this is to reduce reliance on animal testing, streamlining and enhancing the efficiency of chemical risk assessments. Their "Green Deal" includes a plan to inspire globally beneficial management of chemicals[30]. While the plan doesn't directly mention heart toxicity, it highlights the importance of assessing chemicals that might harm specific organs - which certainly includes the heart. In addition, the 2027 vision of the European Food Safety Authority (EFSA) explicitly foresees to develop and integrate non-animal-based methods for regulatory risk assessment[31]

ADVANCEMENTS IN NON-ANIMAL METHODS FOR CARDIOTOXICITY EVALUATION

Traditional animal-based studies often fall short in translating findings to human implications. Thus, Non-Animal Methods are emerging as alternatives. Harnessing methods like in vitro and in silico provides us with data that can be both scientifically robust and more pertinent to human health. This section addresses the current advances of innovative methods using human cells in vitro to evaluate cardiotoxicity, avoiding the need for animal testing.

Electrophysiological/ Proarrhythmic Cardiotoxicity

Chemicals can significantly affect heart rhythms, leading to harmful arrhythmias. Current assays based on the hERG channel, have shown in-consistencies in their clinical relevance. Instead, the use of human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), in both 2D and 3D models[32 33 34 35 36 37], offers a more direct understanding of how chemicals interact with human heart rhythms. Additionally, integrating in vitro findings with in silico data holds promise in forecasting the potential effects of drugs on human cardiac systems[38].

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[29]Commission, E. *Chemicals strategy: The EU's chemicals strategy for sustainability towards a toxic-free environment*. Jun. 06, 2023]; Available from: https://ec.europa.eu/environment/strategy/chemicals-strategy_en

[30]Commission, E. *A European Green Deal*. Available from: https://ec.europa.eu/info/strategy/priorities-2019-2024/european-green-deal_en

[31](EFSA), E.F.S.A., *EFSA Strategy 2027: Science, Safe food, Sustainability.* 2021.

[32]Blinova, K., et al., *Comprehensive Translational Assessment of Human-Induced Pluripotent Stem Cell Derived Cardiomyocytes for Evaluating Drug-Induced Arrhythmias.* Toxicol Sci, 2017. **155**(1): p. 234-247.

[33]Pfeiffer-Kaushik, E.R., et al., *Electrophysiological characterization of drug response in hSC-derived cardiomyocytes using voltage-sensitive optical platforms.* J Pharmacol Toxicol Methods, 2019. **99**: p. 106612

[34]Ando, H., et al., *A new paradigm for drug-induced torsadogenic risk assessment using human iPS cell-derived cardiomyocytes.* J Pharmacol Toxicol Methods, 2017. **84**: p. 111-127.

[35]da Rocha, A.M., et al., *Detection of Drug-Induced Torsades de Pointes Arrhythmia Mechanisms Using hiPSC-CM Syncytial Monolayers in a High-Throughput Screening Voltage Sensitive Dye Assay.* Toxicol Sci, 2020. **173**(2): p. 402-415.

[36]Lu, H.R., et al., *Assessing Drug-Induced Long QT and Proarrhythmic Risk Using Human Stem-Cell-Derived Cardiomyocytes in a Ca2+ Imaging Assay: Evaluation of 28 CiPA Compounds at Three Test Sites.* Toxicol Sci, 2019. **170**(2): p. 345-356.

[37]Kofron, C.M., et al., *A predictive in vitro risk assessment platform for pro-arrhythmic toxicity using human 3D cardiac microtissues.* Sci Rep, 2021. **11**(1): p. 10228.

[38]Shi, M., et al., *A new approach methodology (NAM) for the prediction of (nor)ibogaine-induced cardiotoxicity in humans.* ALTEX, 2021. **38**(4): p. 636-652.

Structural Cardiotoxicity

Chemicals can affect the very structure of our heart by causing morphological damage and sub-cellular damage in cardio-myocytes. In severe or chronic cases, this can lead to cardiac remodeling, characterized by hypertrophy and fibrosis, resulting in heart failure. Recognizing this, there is a shift towards using hiPSC-CMs for high-content screening to unveil potential threats. Advanced 3D cardiac microtissue models, composed of a variety of heart-related cells, stand at the cutting edge[39] [40] [41].

Contractile Cardiotoxicity

The heart's contractile function is vital to its role. To detect threats to this mechanism, research has innovated techniques leveraging hiPSC-CMs[42], machine learning[43], and 3D cardiac micro-tissue models[44] [45]. These methods aim to shed light on how various chemical stressors can modify the heart's essential contractile properties.

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[39]Archer, C.R., et al., *Characterization and Validation of a Human 3D Cardiac Microtissue for the Assessment of Changes in Cardiac Pathology.* Sci Rep, 2018. **8**(1): p. 10160.

[40]Rampoldi, A., et al., *Cardiac Toxicity From Ethanol Exposure in Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes.* Toxicol Sci, 2019. **169**(1): p. 280-292.

[41]Verheijen, M., et al., *Bringing in vitro analysis closer to in vivo: Studying doxorubicin toxicity and associated mechanisms in 3D human microtissues with PBPK-based dose modelling.* Toxicol Lett, 2018. **294**: p. 184-192.

[42]Sirenko, O., et al., *Assessment of beating parameters in human induced pluripotent stem cells enables quantitative in vitro screening for cardiotoxicity.* Toxicol Appl Pharmacol, 2013. **273**(3): p. 500-7.

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