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Review Article

Pollutant Exposure and Myocardial Injury: Protocol and Progress Report for a Toxicological Systematic Mapping Review

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Abstract

An increasing body of evidence identifies pollutant exposure as a risk factor for cardiovascular disease (CVD), while CVD incidence rises steadily with the aging population. Although numerous experimental studies are now available, the mechanisms through which lifetime exposure to environmental pollutants can result in CVD are not fully understood. To comprehensively describe and understand the pathways through which pollutant exposure leads to cardiotoxicity, a systematic mapping review of the available toxicological evidence is needed. This protocol outlines a step-by-step framework for conducting this review. Using the National Toxicology Program (NTP) Health Assessment and Translation (HAT) approach for conducting toxicological systematic reviews, we selected 362 out of 8111 *in vitro* (17%), *in vivo* (67%), and combined (16%) studies for 129 potential cardiotoxic environmental pollutants, including heavy metals (29%), air pollutants (16%), pesticides (27%), and other chemicals (28%). The internal validity of included studies is being assessed with HAT and SYRCLE Risk of Bias tools. Tabular templates are being used to extract key study elements regarding study setup, methodology, techniques, and (qualitative and quantitative) outcomes. Subsequent synthesis will consist of an explorative meta-analysis of possible pollutant-related cardiotoxicity. Evidence maps and interactive knowledge graphs will illustrate evidence streams, cardiotoxic effects and associated quality of evidence, helping researchers and regulators to efficiently identify pollutants of interest. The evidence will be integrated in novel Adverse Outcome Pathways to facilitate regulatory acceptance of non-animal methods for cardiotoxicity testing. The current article describes the progress of the steps made in the systematic mapping review process.

Plain language summary

Heart disease is a leading global cause of death. Recent research indicates that certain environmental chemicals can worsen heart problems. We're conducting a rigorous review of scientific studies to understand how these chemicals affect the heart. This will inform policymakers and promote non-animal testing methods for cardiotoxicity by providing a clear overview of the toxicological evidence. We have reviewed over 8,000 articles and focused on 362 studies about 129 chemicals, including heavy metals, air pollutants and pesticides, and their effects on the heart. The current manuscript describes the used methods and steps made in this process. The outcome of our systematic review of these 362 articles will be a comprehensive database that will aid the development of alternative testing methods for cardiotoxicity.

1 Introduction

Humans are continuously exposed to a vast amount and variety of potentially harmful substances that are ubiquitously present in the surrounding environment. Exposure to these environmental chemicals and their mixtures represents a source of concern due to their multi-organ damaging potential. Cardiovascular disease (CVD) is the leading cause of death globally, and the incidence of CVD is expected to rise with the aging population as age poses the largest risk factor for CVD (WHO, 2019; North and Sinclair, 2012). Additionally, exposure to environmental chemicals could be an important risk factor contributing to the development and

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severity of CVD (Cosselman et al., 2015). Indeed, several epidemiological studies and systematic reviews have linked the exposure to pollutants such as particulate matter (Du et al., 2016), pesticides (Georgiadis et al., 2018), heavy metals (Agarwal et al., 2011), bisphenol A (Lang et al., 2008; Melzer et al., 2010; Melzer et al., 2012), polycyclic aromatic hydrocarbons (Clark et al., 2012), nanoparticles (Donaldson et al., 2013), and persistent organic pollutants (Lind and Lind, 2012) to CVD outcomes including hypertension, coronary heart disease, stroke, and heart failure (Burroughs Peña and Rollins, 2017; GBD 2019 Risk Factors Collaborators, 2020).

Yet, existing regulatory guidelines fall short in evaluating possible cardiotoxicity caused by pharmaceuticals, and even more so by chemicals, biocides, and pesticides that humans may come into contact within the workplace, via food, or in the environment (Schaffert et al., 2023; Daley et al., 2023). For pharmaceuticals, these guidelines largely rely on animal studies, which suffer from animal to human interspecies differences, and *in vitro* methods which do not comprehensively cover all relevant modes of action (MoA). As for chemicals, biocides and pesticides, the guidelines hardly include any specific endpoints for cardiotoxicity. Consequently, potential cardiotoxicity is either not well characterized or it is rarely immediately apparent for regulators. The latter leads to a lack of interest of regulators to investigate the potential cardiotoxic risks for chemicals, pesticides and biocides, resulting in a catch-22.

New Approach Methodologies (NAMs) are emerging tools in regulatory toxicology that aim to inform chemical risk assessment by (integration of) *in vitro*, *ex vivo* and/or *in silico* methods, reducing and ultimately eliminating the need for conventional animal studies (van der Zalm et al., 2022). NAMs may replicate the biological processes of humans, including susceptible populations, and thus provide mechanistic information on toxicity in humans. Additionally, NAMs may provide information on molecular and cellular burdens that reduce the capacity of an organism to cope with the variable additional stress from real-life and environment, which can hardly be comprehensively tested in any conventional test system. As such, NAMs are expected to become the basis for future regulations, which may be more human relevant and practical in terms of costs, time, and ethics. Thereby, the current catch-22 may be overcome and knowledge about and understanding of the potential cardiotoxicity of chemicals may increase (NASEM, 2022; van der Zalm et al., 2022). Systematic reviews can provide a foundation for regulatory action and for the development of such NAMs by offering a transparent and actionable overview of the current toxicological evidence and knowledge gaps.

Adverse Outcome Pathways (AOPs) can inform and guide the development of NAMs by providing mechanistic insight in toxicity pathways. AOPs are conceptual frameworks that describe a sequential chain of causally linked key events (KEs) from a molecular initiating event (MIE) to an adverse outcome (AO), coupled by key event relationships (KERs) through different levels of biological organization. AOPs are useful for the systematic collection and integration of available information about the potential toxicity of chemicals based on *in vitro* molecular-cellular data, *in vivo* animal data, and human clinical and epidemiology data (Ankley et al., 2010; Leist et al., 2017). Therefore, knowledge assessed with AOPs has a dual function: First, it supports the comprehensive understanding and presentation of the currently available toxicological evidence, and thereby helps to provide the evidence of a human health concern from cardiotoxicity of chemicals. Second, it helps to establish the regulatory relevance of future NAM-based assessment approaches, which may subsequently also support the evolution of current legislation towards the use of NAMs for hazard classification and risk assessment (Bajard et al., 2023). Both of these two functions are key to overcome the current catch-22 in regulatory action. By utilizing systematic reviews in AOP development, the required mechanistic information is systematically collected and assessed, enhancing the overall transparency and scientific quality of the AOP and its use within NAMs (De Vries et al., 2021). The evidence on cardiotoxicity of environmental pollutants can be incorporated into AOP networks, making knowledge and concerns transparent and actionable, thereby promoting the regulatory utilization of NAMs.

Currently, numerous NAMs for cardiotoxicity exist that mainly focus on detecting acute effects of pharmaceuticals on the heart (Daley et al., 2023). However, environmental pollutants encompass a much broader chemical space than pharmaceuticals, and their mechanisms of toxicity and potencies are also substantially different. Considering the ever-increasing number of environmental chemicals, there is a pressing need for NAMs that can efficiently assess their cardiotoxic potential. An example of such a NAM currently in development is the ALTERNATIVE[1] project (environmentAL Toxicity chEmical mixtuRes through aN innovative platform based on aged cardiac tissue models), funded by the EU's research and innovation Horizon 2020 program (Grant agreement ID: 101037090), which employs a 5D (3D cell culture, time, and computational methods) human-induced pluripotent stem cell (hiPSC)-based microphysiological human heart model. In the ALTERNATIVE project, the focus is on detecting cardiotoxicity by mimicking the heart physiology in a bioreactor setup, which allows for the detection of endpoints that are closely related to contractility and heart failure. Such NAMs are needed to efficiently characterize the full cardiotoxic potential of environmental pollutants without the use of animals.

By providing an overview of the current toxicological knowledge and mechanistic insight in the predominant pathways of Pollutant Induced CardioToxicity (PICT), this systematic mapping review aims to support and inform the further development of cardiotoxicity AOPs and other NAMs, such as in the H2020 ALTERNATIVE project.

[1] https://alternative-project.eu/

2 Materials and methods

2.1 Methods used

2.1.1 Objective

This protocol sets out the development of a methodological step-by-step framework for a systematic mapping review that aims to systematically characterize and analyze the cardiotoxic potential of environmental pollutants. Evidence maps and knowledge graphs will illustrate evidence streams, cardiotoxic effects and associated quality of evidence for pollutants, helping researchers and regulators to efficiently identify pollutants of interest. Explorative meta-analyses will be performed for a subset of predominantly studied pollutants to investigate overall cardiotoxic effects and to compare evidence streams. Additionally, the systematic mapping review will provide input for the development of a PICT-AOP in support of the regulatory acceptance of NAMs such as aimed for in the ALTERNATIVE project.

2.1.2 Search Strategy and Information Sources

The search terms followed the Population-Exposure-Comparator-Outcome (PECO) format and included a population component (laboratory animals *(in/ex vivo)* and human or animal tissues or cells *(in vitro)*, an exposure component (environmental pollutants), and an outcome component (cardiotoxicity). These three components were combined using the Boolean operator "*AND"*. Following the Handbook for Conducting Systematic Reviews for Health Effect Evaluations (NTP) (NTP, 2019), we searched PubMed, Embase, Web of science and Scopus databases for relevant publications. We started with search development for PubMed. For this database, search terms comprised Medical Subject Headings (MeSH) and title/abstract/keyword terms for each component, in order to retrieve both indexed and non-indexed literature. The PubMed search was validated by an independent expert and subsequently translated for EMBASE, Web of Science, and Scopus using Bond University's validated Polyglot Systematic Review Accelerator (Clark et al., 2020). The resulting search strings were tested string by string (i.e., for each PECO component) in each database, and minor syntax changes were made where necessary. Final search strings can be found in Table 1. The searches were all conducted on August 15th, 2022.

Tab. 1: Search strategy, including number of retrieved references on August 15, 2022

Database	Population	Exposure	Outcome	No. of results
PubMed	("in vitro"[Tiab] OR "cell model"[Tiab] OR "myocard*"[Tiab] OR "cardiomyocyte*"[Tiab] OR "cardiac myocyt*"[Tiab] OR "PSC"[Tiab] or "CMs"[Tiab] OR "endothelial cell*"[Tiab] OR "HCAEC*"[Tiab] OR "AC16"[Tiab] OR "HL-1"[Tiab] OR "H9C2"[Tiab] OR "animal experimentation"[Mesh] OR "models, animal"[Mesh] OR "Animals"[Mesh:noexp] OR "vivo"[Tiab])	("Agrochemicals"[Mesh] OR "Disinfectants"[Mesh] OR "Flame retardants"[Mesh] OR "Lubricants"[Mesh] OR "Plasticizers"[Mesh] OR "Endocrine disruptors"[Mesh] OR "Environmental pollutants"[Mesh] OR "Cardiotoxins"[Mesh] OR "Environmental pollution"[Mesh] OR "Hazardous substances"[Mesh] OR "Pesticides"[Mesh] OR "vehicle emissions"[Mesh] OR "Insecticid*"[Tiab] OR "Pesticid*"[Tiab] OR "Herbicid*"[Tiab] OR "Fungicid*"[Tiab] OR "Chemical*"[Tiab] OR "Flame retardant*"[Tiab] OR "Pollut*"[Tiab] OR "Exhaust particle*"[Tiab] OR "DEP"[Tiab] OR "Particulate matter*"[Tiab] OR "Particulate matter"[Mesh] OR "Air particle*"[Tiab] OR "PM2.5"[Tiab] OR "PM5"[Tiab] OR "PM10"[Tiab] OR "POP"[Tiab] OR "Contamina*"[Tiab] OR "Heavy metal*"[Tiab] OR "Arsenic"[Tiab] OR "cadmium"[Tiab] OR "mercury"[Tiab] OR "chromium"[Tiab] OR "CrVI"[Tiab] OR "lead exposure"[Tiab] OR "Solvent*"[Tiab] OR "Bisphenol*"[Tiab] OR "Dioxin*"[Tiab] OR "PCB"[Tiab] OR "hydrocarbon*"[Tiab] OR "Perfluor*"[Tiab] OR "Polyfluor*"[Tiab] OR "Fluorinated"[Tiab] OR "Fluorocarbon"[Tiab] OR "Halogenated"[Tiab] OR "Brominated" [Tiab])	("cardiotoxicity"[Mesh] OR "cardiomyopathies"[Mesh] OR "heart failure*"[Tiab] OR "Heart failure"[Mesh] OR "cardiotox*"[Tiab] OR "cardio* tox*"[Tiab] OR "cardiac tox*"[Tiab] OR "heart tox*"[Tiab] OR "cardiomyopath*"[Tiab] OR "cardiac myopath*" [Tiab] OR "cardiac hypertrophy"[Tiab])	k = 2880
EMBASE	('in vitro':ti,ab OR 'cell model':ti,ab OR cardiomyocyte*:ti,ab OR 'cardiac myocyt*':ti,ab OR PSC:ti,ab OR CMs:ti,ab OR 'endothelial cell*':ti,ab OR HCAEC*:ti,ab OR AC16:ti,ab OR HL-1:ti,ab OR H9C2:ti,ab OR 'animal experimentation'/exp OR 'models,	(Agrochemicals/exp OR Disinfectants/exp OR 'Flame retardants'/exp OR Lubricants/exp OR Plasticizers/exp OR 'Endocrine disruptors'/exp OR 'Environmental pollutants'/exp OR Cardiotoxins/exp OR 'Environmental pollution'/exp OR 'Hazardous substances'/exp OR Pesticides/exp OR 'vehicle emissions'/exp OR Insecticid*:ti,ab OR Pesticid*:ti,ab OR Herbicid*:ti,ab OR Fungicid*:ti,ab OR 'Flame retardant*':ti,ab OR Pollut*:ti,ab OR 'Exhaust particle*':ti,ab OR DEP:ti,ab OR 'Particulate matter*':ti,ab OR 'Particulate matter'/exp OR 'Air particle*':ti,ab OR PM2.5:ti,ab OR PM5:ti,ab OR PM10:ti,ab OR POP:ti,ab OR Contamina*:ti,ab OR 'Heavy metal*':ti,ab OR Arsenic:ti,ab OR cadmium:ti,ab OR mercury:ti,ab OR chromium:ti,ab OR CrVI:ti,ab OR 'lead exposure':ti,ab OR Solvent*:ti,ab OR Bisphenol*:ti,ab OR Dioxin*:ti,ab OR	(cardiotoxicity/de OR cardiomyopathies/de OR 'heart failure*':ti,ab OR 'Heart failure'/exp OR cardiotox*:ti,ab OR 'cardio tox*':ti,ab OR 'cardiac tox*':ti,ab OR 'heart tox*':ti,ab OR cardiomyopath*:ti,ab OR 'cardiac	k = 4556

	animal'/exp OR Animals/de OR vivo:ti,ab)	PCB:ti,ab OR hydrocarbon*:ti,ab OR Perfluor*:ti,ab OR Polyfluor*:ti,ab OR Fluorinated:ti,ab OR Fluorocarbon:ti,ab OR Halogenated:ti,ab OR Brominated:ti,ab)	myopath*':ti,ab OR 'cardiac hypertrophy':ti,ab)	
Web of science	((TI="in vitro" OR AB="in vitro") OR (TI="cell model" OR AB="cell model") OR (TI=myocard* OR AB=myocard*) OR (TI=cardiomyocyte* OR AB=cardiomyocyte*) OR (TI="cardiac myocyt*" OR AB="cardiac myocyt*") OR (TI=PSC OR AB=PSC) OR (TI=CMs OR AB=CMs) OR (TI="endothelial cell*" OR AB="endothelial cell*") OR (TI=HCAEC* OR AB=HCAEC*) OR (TI=AC16 OR AB=AC16) OR (TI=HL-1 OR AB=HL-1) OR (TI=H9C2 OR AB=H9C2) OR ALL="animal experimentation" OR ALL="models, animal" OR ALL=Animals OR (TI=vivo OR AB=vivo))	(ALL=Agrochemicals OR ALL=Disinfectants OR ALL="Flame retardants" OR ALL=Lubricants OR ALL=Plasticizers OR ALL="Endocrine disruptors" OR ALL="Environmental pollutants" OR ALL=Cardiotoxins OR ALL="Environmental pollution" OR ALL="Hazardous substances" OR ALL=Pesticides OR ALL="vehicle emissions" OR (TI=Insecticid* OR AB=Insecticid*) OR (TI=Pesticid* OR AB=Pesticid*) OR (TI=Herbicid* OR AB=Herbicid*) OR (TI=Fungicid* OR AB=Fungicid*) OR (TI=Chemical* OR AB=Chemical*) OR (TI="Flame retardant*" OR AB="Flame retardant*") OR (TI=Pollut* OR AB=Pollut*) OR (TI="Exhaust particle*" OR AB="Exhaust particle*") OR (TI=DEP OR AB=DEP) OR (TI="Particulate matter*" OR AB="Particulate matter*") OR ALL="Particulate matter" OR (TI="Air particle*" OR AB="Air particle*") OR (TI=PM2.5 OR AB=PM2.5) OR (TI=PM5 OR AB=PM5) OR (TI=PM10 OR AB=PM10) OR (TI=POP OR AB=POP) OR (TI=Contamina* OR AB=Contamina*) OR (TI="Heavy metal*" OR AB="Heavy metal*") OR (TI=Arsenic OR AB=Arsenic) OR (TI=cadmium OR AB=cadmium) OR (TI=mercury OR AB=mercury) OR (TI=chromium OR AB=chromium) OR (TI=CrVI OR AB=CrVI) OR (TI="lead exposure" OR AB="lead exposure") OR (TI=Solvent* OR AB=Solvent*) OR (TI=Bisphenol* OR AB=Bisphenol*) OR (TI=Dioxin* OR AB=Dioxin*) OR (TI=PCB OR AB=PCB) OR (TI=hydrocarbon* OR AB=hydrocarbon*) OR (TI=Perfluor* OR AB=Perfluor*) OR (TI=Polyfluor* OR AB=Polyfluor*) OR (TI=Fluorinated OR AB=Fluorinated) OR (TI=Fluorocarbon OR AB=Fluorocarbon) OR (TI=Halogenated OR AB=Halogenated) OR (TI=Brominated OR AB=Brominated))	(ALL=cardiotoxicity OR ALL=cardiomyopathies OR (TI="heart failure*" OR AB="heart failure*") OR ALL="Heart failure" OR (TI=cardiotox* OR AB=cardiotox*) OR (TI="cardio tox*" OR AB="cardio tox*") OR (TI="cardiac tox*" OR AB="cardiac tox*") OR (TI="heart tox*" OR AB="heart tox*") OR (TI=cardiomyopath* OR AB=cardiomyopath*) OR (TI="cardiac myopath*" OR AB="cardiac myopath*") OR (TI="cardiac hypertrophy" OR AB="cardiac hypertrophy"))	k = 2066
Scopus	(TITLE-ABS("in vitro") OR TITLE-ABS("cell model") OR TITLE-ABS(cardiomyocyte*) OR TITLE-ABS("cardiac myocyt*") OR TITLE-ABS(PSC) OR TITLE-ABS(CMs) OR TITLE-ABS("endothelial cell*") OR TITLE-ABS(HCAEC*) OR TITLE-ABS(AC16) OR TITLE-ABS(HL-1) OR TITLE-ABS(H9C2) OR INDEXTERMS("animal experimentation") OR INDEXTERMS("models, animal") OR INDEXTERMS(Animals) OR TITLE-ABS(vivo))	(INDEXTERMS(Agrochemicals) OR INDEXTERMS(Disinfectants) OR INDEXTERMS("Flame retardants") OR INDEXTERMS(Lubricants) OR INDEXTERMS(Plasticizers) OR INDEXTERMS("Endocrine disruptors") OR INDEXTERMS("Environmental pollutants") OR INDEXTERMS(Cardiotoxins) OR INDEXTERMS("Environmental pollution") OR INDEXTERMS("Hazardous substances") OR INDEXTERMS(Pesticides) OR INDEXTERMS("vehicle emissions") OR TITLE-ABS(Insecticid*) OR TITLE-ABS(Pesticid*) OR TITLE-ABS(Herbicid*) OR TITLE-ABS(Fungicid*) OR TITLE-ABS("Flame retardant*") OR TITLE-ABS(Pollut*) OR TITLE-ABS("Exhaust particle*") OR TITLE-ABS(DEP) OR TITLE-ABS("Particulate matter*") OR INDEXTERMS("Particulate matter") OR TITLE-ABS("Air particle*") OR TITLE-ABS(PM2.5) OR TITLE-ABS(PM5) OR TITLE-ABS(PM10) OR TITLE-ABS(POP) OR TITLE-ABS(Contamina*) OR TITLE-ABS("Heavy metal*") OR TITLE-ABS(Arsenic) OR TITLE-ABS(cadmium) OR TITLE-ABS(mercury) OR TITLE-ABS(chromium) OR TITLE-ABS(CrVI) OR TITLE-ABS("lead exposure") OR TITLE-ABS(Solvent*) OR TITLE-ABS(Bisphenol*) OR TITLE-ABS(Dioxin*) OR TITLE-ABS(PCB) OR TITLE-ABS(hydrocarbon*) OR TITLE-ABS(Perfluor*) OR TITLE-ABS(Polyfluor*) OR TITLE-ABS(Fluorinated) OR TITLE-ABS(Fluorocarbon) OR TITLE-ABS(Halogenated) OR TITLE-ABS(Brominated))	(INDEXTERMS(cardiotoxicity) OR INDEXTERMS(cardiomyopathies) OR TITLE-ABS("heart failure*") OR INDEXTERMS("Heart failure") OR TITLE-ABS(cardiotox*) OR TITLE-ABS("cardio tox*") OR TITLE-ABS("cardiac tox*") OR TITLE-ABS("heart tox*") OR TITLE-ABS(cardiomyopath*) OR TITLE-ABS("cardiac myopath*") OR TITLE-ABS("cardiac hypertrophy"))	k = 2065

Tab. 2: Eligibility criteria per study element

Study element	Inclusion criteria	Exclusion criteria
Populations	*In vitro* or *in/ex vivo* studies that utilize healthy species relevant for studying cardiotoxicity, including cardiomyocyte studies, heart perfusion studies, and other non-developmental animal studies.	Studies that are not performed *in vitro* or *in/ex vivo*, or studies that use species/cell types not relevant for assessing cardiotoxicity in healthy subjects, comprising e.g., disease models focusing on comorbidities.
Exposures	Environmental pollutants and contaminants, or substances to which exposure is likely to occur in a population though environmental exposure, including pesticides, heavy metals, and air pollutants.	Substances that are not considered environmental pollutants, chemical mixtures with unknown composition or no exposure studied.
Study design	Primary study designs that are able to clearly and solely link the exposure of interest to the outcome of interest with an appropriate control condition.	Study designs unable to exclusively assess cardiotoxic effects for only the exposure of interest (e.g., ischemia/reperfusion studies, studies with multiple treatments in the group of interest), meta-research.
Publication type	Full text, accepted peer reviewed manuscripts.	Publications that are (systematic) reviews, abstract-only, or articles that are not peer reviewed, published in a (potential) predatory journal as identified in Beall's list[9], or not online accessible to the core review team [TR, SM, AS].
Language	Articles written in English.	Articles written in any language other than English.
Outcomes	Outcomes that are directly associated with contractile dysfunction or that can lead to contractile dysfunction as a proxy for heart failure, including hypertrophy, cytotoxicity, disrupted contractility, cardiomyopathy, reduced cardiac output.	Outcomes that are not relevant for inducing or contributing to contractile dysfunction.

The population component was used without restrictions for species or cell type. The exposure component included several environmental (air) pollutants (particulate matter, persistent organic pollutants, soil and water pollutants), pesticides, heavy metals, polycyclic compounds, and substances that are (suspected) contaminants of the natural environment and/or substances in the environment to which exposure is likely to occur. A comparator component was not used in the search as we aimed to include all comparators and it would have limited the number of retrieved studies. The outcome component entailed cardiotoxicity, defined in this review as the mechanical disruption of the heart muscle, and therefore we included endpoints that could contribute to this phenotype in our search string; including cardiomyopathy, disrupted contractility, ventricular and atrial dysfunction, cytotoxic effects in the cardiomyocytes, reduced cardiac output and heart failure.

2.1.3 Eligibility Criteria

The studies retrieved from our searches were assessed based on predefined inclusion and exclusion criteria, used for the title and abstract screening, summarized in Table 2.

2.1.4 Title/abstract review, full text review

Two investigators (TR, SM) independently screened all references at the title and abstract level in a double blind fashion using Rayyan[2], a free web-tool designed to help researchers working on systematic reviews speeding up the process of screening and selecting studies (Ouzzani et al., 2016). References were not considered further when it was clear from the title or abstract that the study did not meet the eligibility criteria. When all eligibility criteria were (potentially) met, the record was marked as 'included'. All discrepancies were resolved by discussion between the investigators; consulting an independent expert was not necessary. After completion of the title/abstract screen, full-text articles were retrieved. Two out of three investigators (TR, SM, AS) performed full text reviews independently in a double blind fashion, and discrepancies were resolved without the need for consulting an independent expert.

2.2 Methods – in progress and planned

2.2.1 Data extraction

Data is currently being extracted from the identified studies in predefined templates that were adapted from the HAT handbook (NTP, 2019), using the web-based application Covidence[3]. An overview of extracted study elements for the various evidence streams can be found in Table 3. Risk of bias (RoB) assessments are being performed during the data extraction stage by using the RoB checklist (Tab. 4). Data relevant for AOP development are being extracted using a specific AOP template (Tab. S1[4]).

[2] https://www.rayyan.ai/
[3] https://www.covidence.org
[4] doi:10.14573/altex.2304111s

Tab. 3*:* Data extraction elements
Listed are elements applicable to both *in vivo* AND *vitro* studies (left), to *in vivo* studies (center) and to *in vitro* studies (right).

In vivo* AND *in vitro	***In vivo***	***In vitro***
COI statement	Sex	Sex of human/animal origin
Guideline compliance	Species	Cell line, type, or tissue, type of culture system
Chemical name	Strain, limited to basic details	Source of cells/tissue, species, strain (basic details)
Chemical class	Age or life stage at start of dosing and at health outcome assessment	Age of cells at start of dosing and at outcome assessment
Composition of chemical mixture	Dose range (mg/kg bw) (min – max)	Concentration range (μM) (min – max)
CAS number(s)	Route of administration	Number of replicates per group
Source of chemical	Number of animals per group	Frequency of dosing
Purity of chemical	Dosing interval	(Total) incubation time
(Negative) control used (vehicle)	Duration/length of exposure scenario	
Outcome measures in study		
Methods used to obtain outcome measures		
Outcome treatment group		
Outcome control group		
SEM treatment group		
SEM control group		
Guideline compliance		

SEM: Standard Error of the Mean. COI: Conflict of Interest.

Tab. 4: Risk of bias (RoB) checklist

Selection bias
1. Was the administered dose or exposure level adequately randomized?
2. Was allocation of animals or cells to study groups adequately concealed?
3. Were the groups similar at baseline or adjusted for confounders?
Performance bias
4. Were experimental conditions identical across study groups?
5. Was the research personnel blinded to the study group during the study?
Attrition/exclusion bias
6. Were outcome data complete without attrition or exclusion from analysis?
Detection bias (key criteria)
7. Were animals selected at random for outcome assessment?
8. To what extent can we expect the exposure characterization to be biased?
9. To what extent can we expect the outcome assessment to be biased?
Selective reporting bias
10. Were all measured outcomes reported?
11. Were there no other potential threats to internal validity?

2.2.2 Study and Evidence Quality Assessment

Individual animal and *in vitro* studies are being assessed for internal validity and risk of bias (RoB) by using a combination of SYRCLE (Hooijmans et al., 2014) and NTP's HAT RoB tools (NTP, 2019). The risk of bias will be evaluated by completing the RoB checklist for every included reference (Tab. 4). Questions on the checklist can be answered with probably low risk of bias, unclear risk of bias, or probably high risk of bias. The checklist will also be applied to *in vitro* evidence. After answering all questions, different tiers of evidence quality can be determined, ranging from tier 1 (low risk of bias) to tier 3 (high risk of bias).

2.3 AOP development

2.3.1 Data extraction

The evidence gathered by the systematic mapping review will be utilized to create a comprehensive AOP network. This network will integrate a wide array of the gathered mechanistic information reflecting the diverse pathways of cardiotoxicity and focusing on the most frequently observed and most essential Key Event (KE) relationships identified in our review. The network will be

based on and expand our previously created putative AOPs 479[5] and 480[6], which are grounded on well-established cardiotoxicity mechanisms (Klaassen, 2018; Hayes and Kruger, 2014), and both describe mitochondrial dysfunction subsequently leading to cardiotoxicity and heart failure. It is well established that mitochondria play a pivotal role in energy production, ensuring a continuous supply of energy for cardiomyocytes, and their toxicity and dysfunction can result in insufficient cardiac output, ultimately leading to heart failure. The mechanistic information will be collected using the AOP data extraction template (Tab. S1[4]). The extraction of information will be conducted according to the guidelines of the OECD Developer's handbook (OECD, 2018).

2.3.2 Evidence assessment

The weight of evidence (WoE) for the overall AOP will be assessed based on Bradford-Hill criteria and the OECD developer's handbook (OECD, 2018; Becker et al., 2015). In brief, the assessment includes three criteria: (1) biological plausibility for KERs, (2) empirical support (dose-response, temporality, and incidence) for KERs, and (3) essentiality of KEs. The criteria are assessed by guiding questions designed to aid the assignment of categories of high, moderate, or low confidence including a brief explanation or justification for the selection (Tab. S2[4]).

2.4 Synthesis of results

2.4.1 Results presented in this paper

A PRISMA study flow diagram was created to visualize the flow of included and excluded references identified, and citing the reasons for exclusion (Fig. 1). During full text screening, included references were categorized by evidence stream *(in vivo/vitro)* and basic study elements were extracted to allow for preliminary evidence mapping. These elements consisted of the animal species and/or cell type used, chemical name, and chemical group, enabling a crosstabulation and preliminary visualization of the amount of evidence for specific cardiotoxicity evidence stream – exposure scenarios (Tab. 6).

2.4.2 Plan for further evidence mapping and meta-analyses

All extracted data will be collated by evidence stream, exposure, and the cardiotoxic MoA. This data will be tabulated and subsequently mapped in evidence maps. These evidence maps and the full dataset, including the data extraction elements from all included studies, will be hosted on Tableau Public (Beard and Aghassibake, 2021). This is a freely accessible online platform in which end users can interactively select and visualize specific exposures and outcomes of interest. The possibility to visualize the weight of evidence and risk of bias will also be included, allowing regulators and researchers to easily identify specific characteristics of pollutants of interest. Successful examples on how to process and implement data from systematic reviews into Tableau Public are available elsewhere (Pelch et al., 2019; Pelch et al., 2022).

The possibilities for informative meta-analyses to assess overall cardiotoxic effects of environmental pollutants were explored after completion of the evidence mapping. Analysis experts blinded to the pollutants decided on a clearcut principle. Blinding was ensured by the creation of a coded crosstabulation. The blinded experts decided that meta-analyses will be performed for two pollutants per chemical group; those with the largest *in vivo* samples (numbers of studies) for which at least k=3 *in vitro* studies are present. This results in planned meta-analyses for cadmium and arsenic (heavy metals), chlorpyriphos and parathion (pesticides), bisphenol A and TCDD (other chemicals), and particulate matter (air pollution).

Meta-analyses will be performed in R[7], via RStudio[8]. Separate random effects meta-analyses will be performed for each pollutant, using standardized mean differences (SMD) to allow for the comparison of outcomes with different scaling in a single analysis. The "metacont" function from the meta package (Balduzzi et al., 2019) will be used for standard between-group comparisons to assess overall effects. Subgroup analyses will be performed to compare different populations (*in vitro, vivo*, species). Results will be visualized in forest plots. The "rma" function from the metafor package (Viechtbauer, 2010) may be used for meta-regressions analyzing dose effects, if different doses are tested in at least 10 separate groups. If meta-regressions can be performed, results will be presented in bubble plots.

For studies that compare more than one dose to a single control group, the number of replicates (n) will be corrected using the following equation: n corrected = n control/number of comparisons. Heterogeneity will be assessed with the I2 statistic (Higgins et al., 2003). If 10 or more studies are included in a meta-analysis, small study effects will be visualized in funnel plots, and their effect will be analyzed with trim-and-fill analysis.

3 Results

An overview of the flow of information through the different phases of this systematic review is depicted in the PRISMA flowchart (Fig. 1). The searches retrieved 12,567 references in total. These references were deduplicated in Endnote Reference Manager prior to uploading the deduplicated database (k = 9112) into the web based Rayyan screening tool[2]. Additional duplicates found in Rayyan (k = 1002) were individually assessed and subsequently deleted or marked as not duplicate, resulting in the final dataset that was screened (k = 8111). After screening, 530 references met the eligibility criteria and were sought for full text retrieval. For 52

[5] https://aopwiki.org/aops/479
[6] https://aopwiki.org/aops/480
[7] https://www.R-project.org/
[8] http://www.posit.co/

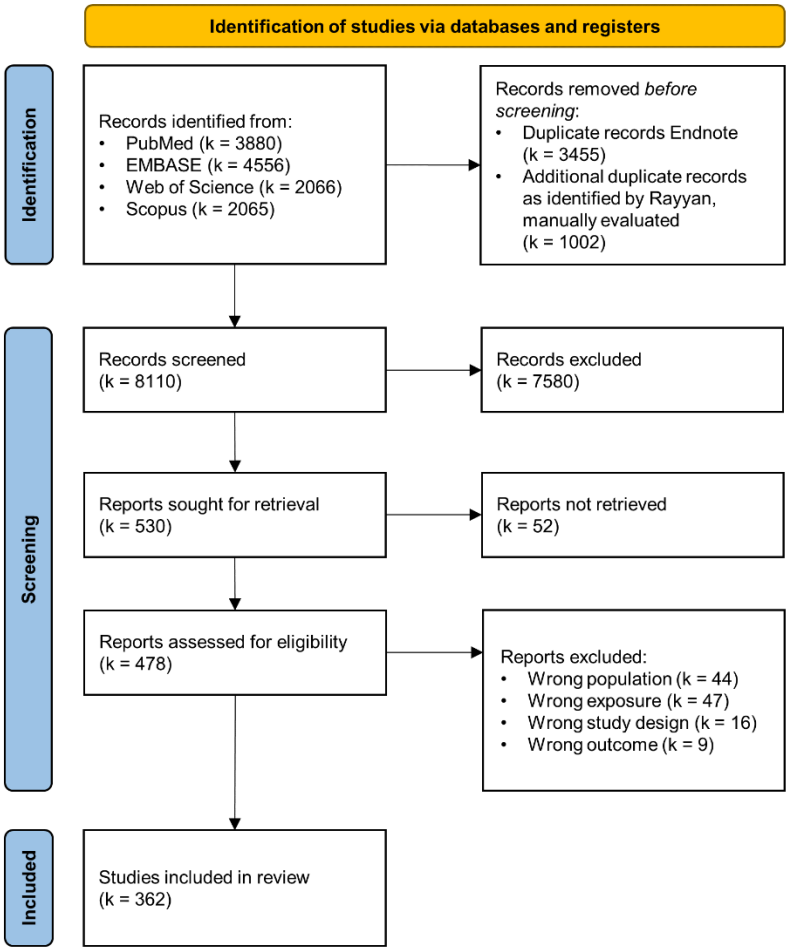

Fig. 1: PRISMA flowchart

references, full text manuscripts were not available or were published in a predatory journal according to Beall's list[9]. In total, 478 references were full-text assessed by the review team after which 362 references were included in this review.

An overview of the number of included references per evidence stream and chemical group can be found in Table 5. The most frequently reported animal models included rats (k = 196) and mice (k = 59), while the most reported cell lines included H9c2 myoblasts (k = 21), cardiomyocytes isolated from rats (k = 19), mice (k = 8), the AC16 human cardiomyocyte cell line (k = 7) and hiPSC-CMs (k = 5).

Overall, included studies described 129 different pollutants. They were ranked by the number of included references, and a shortlist was created for 25 pollutants for which most evidence was available (Tab. 6).

4 Discussion

Toxicological assays can be performed in different test systems, ranging from cell cultures to organs on chips and whole animals, generating large amounts of complex data. The systematic assessment of these studies is a monumental task, especially considering the heterogeneity in this field. Although the use of systematic reviews in the field of toxicology is still emerging, numerous guidance documents and protocols have now been published, highlighting the potential for these reviews in generating high quality evidence. Regarding cardiotoxicity, there is an increasing body of evidence suggesting a positive association between the exposure to environmental pollutants and CVD. However, the mechanisms by which these effects are induced through environmental pollutant exposure remain elusive. Although many mechanistic studies on cardiotoxicity have now been published, an extensive and systematic review that categorizes and characterizes these pollutants and pathways is not available. This synthesis of toxicological evidence is needed to allow for the characterization of PICT, updating regulatory frameworks for cardiotoxicity assessment, and the development of non-animal test methods for cardiotoxicity such as in the ALTERNATIVE project.

[9] Beall's list: Potential, possible, or probable predatory scholarly open-access publishers. https://beallslist.net (accessed 31-03-2023)

Tab. 5: Evidence map of included references, cross tabulated by evidence stream and chemical class

Chemical group	Number of references included per evidence stream			
	in vitro	*in vivo*	combined	**Total**
Air pollutants	9	35	13	**57**
Heavy metals	8	81	16	**105**
Pesticides	12	66	19	**97**
Other chemicals	33	63	7	**103**
Total	62	245	55	**362**

Tab. 6: Top 25 pollutants ranked by total number of included references, categorized by evidence stream
The names of the chemicals selected for future meta-analyses are indicated in **bold**.

Chemical name	Chemical group	Nr. of references included (per evidence stream)			
		vitro	*vivo*	comb.	**Total**
Cadmium	Heavy metals	4	35	4	**43**
Bisphenol A	Other chemicals	3	15	3	**21**
PM2.5	Air pollutants	7	10	3	**20**
Arsenic	Heavy metals	0	13	4	**17**
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	Other chemicals	6	10	0	**16**
Aluminium phosphide	Pesticides	4	9	1	**14**
Diazinon	Pesticides	1	11	1	**13**
Lead	Heavy metals	1	8	3	**12**
Sodium arsenite	Heavy metals	0	11	1	**12**
Paraquat	Pesticides	0	4	6	**10**
Chlorpyrifos	Pesticides	2	5	2	**9**
Diesel exhaust particle (DEP)	Air pollutants	0	6	2	**8**
Mercury	Heavy metals	0	7	1	**8**
Endosulfan	Pesticides	0	5	1	**6**
Acrolein	Other chemicals	1	3	1	**5**
Chromium	Heavy metals	1	3	1	**5**
Di(2-ethylhexyl)phthalate (DEHP)	Other chemicals	1	4	0	**5**
Atrazine	Pesticides	0	4	0	**4**
Carbon monoxide (CO)	Air pollutants	0	3	1	**4**
Malathion	Pesticides	2	2	0	**4**
Ozone	Air pollutants	0	4	0	**4**
Parathion	Pesticides	0	1	3	**4**
Perfluorooctane sulfonate (PFOS)	Other chemicals	3	1	0	**4**
Phenanthrene	Other chemicals	3	0	1	**4**
Tebuconazole	Pesticides	1	2	1	**4**

Multiple NAMs for cardiotoxicity testing currently exist. In general, these primarily focus on electrophysiological effects caused by short term (acute) exposure to pharmaceuticals (Gintant et al., 2020; Sharma et al., 2018; Magdy et al., 2018; Zwartsen et al., 2019) or study specific cellular responses such as calcium flux and cell viability in 2D fashion (Sirenko et al., 2017). Within regulatory guidelines, assays such as these could potentially be used for pre-clinical cardiotoxicity screening. Indeed, efforts are currently underway to consider recognizing cardiotoxicity as a separate hazard class within regulatory frameworks for chemical assessment, which could ensure a more adequate assessment of cardiotoxicity (Georgiadis et al., 2022). For the adoption of NAMs, it might be necessary to explore alternative frameworks and introduce new classification systems. Within regulatory toxicity testing, there is a need for broader *in vitro* endpoints in addition to the well-defined electrophysiological effects in order to make the link with *in vivo* manifestations of cardiotoxicity (Daley et al., 2023). In this regard, assays that can assess contractile, structural, and other functional effects are needed for integration with validated electrophysiological assays to be able to provide regulators with the complex information they need.

To overcome some of these challenges, the ALTERNATIVE project uses a human relevant 3D cell system consisting of hiPSC-CMs and human coronary artery endothelial cells (HCAECs) co-cultured in sensorized bioreactors. The project aims to construct a microphysiological heart system as part of a NAM for cardiotoxicity testing. Despite the great potential for efficient, accurate and animal-free human cardiotoxicity assessment, chemical legislation is currently limited in its regulatory acceptance of such non-animal methods (Westmoreland et al., 2022). To assist in this challenge, this review provides an evidence-based and transparent overview of the available toxicological evidence for PICT. This evidence will be integrated within novel AOPs,

supporting the development of an integrated approach to testing and assessment (IATA) for cardiotoxicity. This IATA is expected to facilitate regulatory acceptance of NAMs for cardiotoxicity assessment in accordance with OECD guidelines (Schaffert et al., 2023).

Environmental pollutant exposure is expected to further exacerbate CVD in vulnerable population groups and in cases where co-exposure to cardiotoxic pharmaceuticals exists. The cardiotoxic potential of regularly used pharmaceuticals such as antineoplastics has been demonstrated extensively in systematic reviews (Alinejad et al., 2015; Shan et al., 1996; Dolci et al., 2008; Schlitt et al., 2014; Orphanos et al., 2009; Cardinale et al., 2020; Ewer and Ewer, 2010, 2015; Guo et al., 2010). Some of these pharmaceuticals exert cardiotoxicity through pathways similar to environmental chemicals, which raises the question whether in some cases there could be synergistic toxicity. The present review and anticipated database could be used to explore synergistic cardiotoxicity pathways based on mechanistic understanding. Furthermore, with cardiotoxicity NAMs such as the ALTERNATIVE project, both pharmaceuticals and environmental chemicals can be efficiently tested for their combined cardiotoxic potential to mimic such (real life) scenarios of combined exposure.

Eventually, results from this systematic mapping review and meta-analyses will be integrated with a review on epidemiological evidence of PICT which is performed in parallel (Linzalone et al., 2022). By doing so, outcomes seen at the population level (heart failure) can be linked to molecular initiating events that start the cardiotoxicity cascade upon exposure, for example, by using the AOP framework.

In conclusion, this systematic mapping review provides a high-level overview of the available toxicological evidence for cardiotoxic effects of environmental pollutants. This is an important step in acknowledging and further assessing the role of environmental pollutants in cardiovascular disease. The protocol for the meta-analyses describes how we will assess and compare the overall effects of selected pollutants, and potential differences between *in vitro* and *in vivo* studies. With the subsequent AOP development, we plan to synthesize results from specific PICT scenarios, elucidate the predominant mechanisms of toxicity, and make the link with the epidemiological evidence, supporting IATA development and regulatory acceptance of non-animal methods for cardiotoxicity testing.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

All available data will be publicly accessible in Tableau Public upon completion of the systematic review. In addition, the data will be published in the ALTERNATIVE Project Zenodo public repository and will be freely accessible to public.