Adverse outcome pathways for chemical toxicity and their applications to workers' health: a literature review

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Abstract

Objective and methods Various papers related to the application of adverse outcome pathways (AOPs) for the prevention of occupational disease were reviewed. The Internet was used as the primary tool to search for the necessary research data and information, using such online resources as Google Scholar, ScienceDirect, Scopus, NDSL, and PubMed. The key search terms were “adverse outcome pathway,” “toxicology,” “risk assessment,” “human,” “worker,” “occupational safety and health,” and so on.

Results and conclusion The aim of this paper is to explain the use of AOP for the understanding of chemical toxicity as a conceptual means and to predict the toxic mechanism. The tools of AOP have emerged as a forward-looking alternative to the existing chemical risk assessment paradigm. AOP is being applied to the assessment of acute toxicity and to chronic toxic chemicals in the workplace. Not only can it lead to breakthroughs in occupational and environmental cancer prevention, it is also widely used in chemical risk assessment and has led to breakthroughs in the prevention of occupational disease in the workplace.

Keywords Adverse outcome pathways · Applications · Chemical toxicity · Review · Workers' health

Abbreviations

ACD	Allergic contact dermatitis	AOP-KB	Knowledge-based AOP
ADME	Absorption, distribution, metabolism, and excretion	LLNAs	Local lymph node assays
AO	Adverse outcome	MoA	Mode of action
AOP	Adverse outcome pathway	MIE	Molecular initiative event
ENMs	Engineered nanomaterials	NRC	National Research Council
EC	European commission	NTP	National Toxicology Program
HCS	High-content screening	NICEATM	NTP interagency center for the evaluation of alternative toxicological methods
HTS	High-throughput screening (or testing)	OELs	Occupational exposure levels
HHRA	Human health risk assessment	OHT	OECD harmonized templates
IATA	Integrated approaches to testing and assessment	OECD	Organization for Economic Cooperation and Development
ITS	Integrated testing strategies	PHMG	Polyhexamethylene guanidine
IEs	Intermediate effects	QSAR	Quantitative structure–activity relationships
IPCS	International Program on Chemical Safety	SARs	Structure–activity relationships
KER	Key event relationship	3D	Three-dimensional
KEs	Key events	EPA	US Environmental Protection Agency
		WoE	Weight of evidence

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Introduction

With the development of modern science and technology, huge quantities of chemicals are used, and more than 15 million chemicals are sold worldwide [1]. Humans routinely use chemicals and can be exposed to them in everyday accidents. Although they are widely used, the impact of many chemicals on human health is still unknown. With increasing concern about the safety of chemicals, human exposure has emerged as an important issue such as humidifier disinfectants. Given the widespread use of chemicals, there are many risks associated with chemical accidents and diseases. Chemical incidents reported worldwide between 1970 and 1998 resulted in leaks, fires, and explosions involving more than 100,000 casualties [2, 3]. Since chemicals are stored in huge quantities, the magnitude and extent of damage when a chemical accident occurs can be significant [2].

In the 2007 “Toxicity test for the twenty-first century: Vision and strategy,” the National Research Council (NRC) planned a new toxicity test system to describe a new paradigm shift in the assessment of the hazards and risks of chemicals, via a new computational biological test method used to study toxic pathways for the expansion of *in vitro* testing [4]. In this context, the “adverse outcome pathway (AOP)” is a biological pathway that disturbs homeostasis, which causes toxicity. The description of toxicity begins with chemical characterization and explains the interaction between them. Target tests to identify impacts at critical stages of the pathway and dose–response extrapolation to estimate human exposure are required. Predicting ecosystem impacts requires additional population-based modeling. This is a conceptual framework for organizing existing biological information, and AOP consists of molecular initiative event (MIE), key events (KEs), and adverse outcome (AO). This independent element represents a biological response and is associated with major event relationships. The AOP concept provides instinctive risk identification to help assess the carcinogenicity of chemicals. This highlights the application of the AOP concept and the effective application of cancer prevention by predicting the carcinogenicity of chemicals [5].

Results

Concept and role in chemical hazard assessment

Molecular initiative event (MIE) is the initial point of the AOP and represents chemical interactions at the molecular

level, such as ligand–receptor binding or DNA binding. Key event (KE) is a biological change caused by a molecular initiative event at various levels, such as cells and tissues. An adverse outcome (AO) is a particular type of KE, representing an endpoint of biological disturbances due to MIE. Molecular initiative event, key event, and AO are connected by a unidirectional arrow called key event relationship (KER), which means predictive interaction between upper and lower KE, making it easy to estimate the state of each KE [6].

Each factor in the AOP must be based on the weight of evidence (WoE) that meets the Bradford Hill criteria. Establishing the mode of action (MoA) hypothesis is based on causality criteria, considerations of coherence, specificity of relationships, coherent time relationships, dose–response relationships, biological validity, coherence of evidence, and alternative explanations [7–9]. The WoE is the concept of providing evidence that meets information requirements using information from independent sources [10]. One AOP should have one MIE and one AO, but there is no limit to the number of intermediate KEs, and each KE can be included in a different AOP. Adverse outcome pathways describe the sequence of causality in biological responses to chemicals at various levels [11–13]. Any type of information related to a biological response (e.g., *in vivo* and *in vitro* test results, electronic data, biomarkers, etc.) may be applied to the AOP [8]. Thus, AOP is a dynamic technology that allows existing information to be more accurately changed as the technology used to monitor biological responses evolves [14]. Because AOP is expressed in a simple flowchart, it is easy to instinctively understand the side effects of chemicals, and the concept of AOP is not a sudden occurrence, but an evolution of existing concepts related to toxic mechanisms [11, 15].

Mode of action (MoA) frameworks

The carcinogen mode of action (MoA) framework of early International Program on Chemical Safety (IPCS) oversees the systematic process of describing the chemical early international programs on chemical MoA in animals and determines relevance in humans through comparisons. Early IPCS techniques set out some basic principles of pathway-based techniques, and MoA is defined as a series of key events along biological pathways from initial chemical interactions to toxicological consequences. One MoA was studied at a time, recognizing that MoA does not have to be complete to be useful; a chemical can have more than one MoA and may therefore be associated with multiple possibilities, each of which can be critical to toxicity studies. To establish a model for defining “key events” as possible pathway steps and to systematically establish causal relationships between key events, the MoA must ensure the importance of setting quantitative parameters. In this regard, the MoA is

distinguished from the mechanism of action, the latter being a more detailed description of pathways involving molecular interactions [16]. The MoA has generally been updated to accommodate insights into the pathway-based approach to risk assessment, where MoA and AOP are conceptually similar, but MoA does not necessarily imply toxic expression (e.g., effect as drugs) [16].

Over the last decade, attempts to describe and predict the biological and toxicological effects of chemicals have gradually shifted to the consideration of mechanized automation. Of the many reports in the burgeoning field of predictive toxicology, the US NRC's "21st Century Toxicity Testing: Vision and Strategy" attracted considerable attention and discussed the need for a paradigm shift in the assessment of the hazards and risks of chemicals [4, 11, 17–21]. The NRC report plans to change the current way of conducting toxicological tests, which are based on animal phenotypes, with techniques that are increasingly dependent on understanding the molecular mechanisms of toxicity in human cells and tissues. The transition to more mechanistic hazard assessment is the "high-throughput screening (HTS) and high-content screening (HCS)" in mammalian (especially human) cell lines and/or tissue cultures. This implies the use of *in vitro* tests based on various calculations for data analysis and interactions between toxicity and molecular targets, side effects, and outcomes. The general premise of AOP technology means that there are sufficient events to explain biological pathways and to predict toxic pathways at different levels of biological tissue (cells, tissues/organs, organisms, populations). A basic understanding of molecular interactions and effects should be able to predict the side effects of organisms and populations.

Adverse outcome pathway technique

The AOP technique was initiated in the field of ecological toxicology as a way to address the uncertainty of hazard assessments for an increasingly diverse environment, and as reported by Ankley et al. [11] adverse outcome pathway is a "causal, mechanical, inferential, or correlated relationship." It is a flexible technology that can include infrastructure-based connections, and the information on which they are based can include both mechanisms and MoA *in vitro*, *in vivo*, or computational systems. Adverse outcome pathway describes an MIE that occurs at the onset of chemical exposure, beginning with the interaction between the biomolecule (e.g., proteins, receptors, etc.) and the molecule and subsequently health in cells and in humans. All types of information can be integrated from the molecular level to the individual level to predict hazard consequences and provide biological arguments (or a set of hypotheses), including individual toxicity levels for hazards or environmental hazards. The Organization for Economic Co-operation and

Development (OECD), the US Environmental Protection Agency (EPA), and the European Commission (EC) joint research centers have developed a chemical knowledge-based toxicity pathway (AOP-KB), and the AOP-KB has attracted the interest and participation of stakeholders, including government, academia, and industry, in that it provides a point of contact with academic research achievements that can be directly linked to government chemical regulation. The concept of AOP is useful for developing predictive toxicity tools, and even short-term imperfect AOPs can show the relationship between chemicals and risk. The certainty of interpretation between existing and new information can be increased, and it can be used as an integrated test strategy to maximize that certainty [22].

Recent trends in adverse outcome pathways

The concept of AOP was first announced in 2010 by Ankley et al. [11] with 243 AOPs and 1800 KEs registered and developing as of August 2018. This AOP information is available free of charge in the AOP-KB, the leading AOP database maintained by the OECD; the site has been published by the US EPA to share and discuss relevant information among AOP developers. The project began in collaboration with EC's joint research center and the US army technician R&D center [23].

Knowledge-based adverse outcome pathway (AOP-KB) consists of AOP-Wiki, Effectopedia, Intermediate Effects DB, and Xplorer [24]. AOP-Wiki provides a system that organizes the available knowledge and published research information concerning AOP, KE, KER, and stressors, respectively. Effectopedia is a modeling platform that is designed for the development and application of AOP. Experimental data are used to provide quantitative information about the AOP component, and a beta version of Effectopedia is currently available. Medium impact DB processes chemical information and describes how chemicals cause MIE or KE. AOP Xplorer is a calculation tool for mapping AOP. Intermediate DB and AOP Xplorer are currently in development, and all four systems share information through the AOP-KB hub. Since each element of AOP-KB is individually arranged, AOP-KB is useful for the development of AOP and is applied from various studies [13]. The OECD provides comprehensive management of AOP development and evaluation [6] and is overseen by the OECD extended advisory group on molecular screening and toxicogenomics (EAGMST). The AOP-Wiki is administered by the Toxic Pathology Society, in accordance with the OECD extended advisory group on molecular screening and toxicogenomics guidelines [23]. Since the OECD published its first AOP, "Skin sensitization pathways initiated by covalent bonds to proteins," several reports and guidance on AOP have been published, and the OECD provides eight AOP-related

publications. As of August 2018, the AOP development program workbook lists 60 projects [24].

AOPs for chemicals in workplace

Respiratory sensitivity is an important occupational health issue for workers, and understanding the mechanistic basis for this effect is necessary to support the development of toxicological tools to detect the chemicals. Recently, AOP systems and techniques have been used to organize the information to better convey the understanding of chemical respiratory sensitization, which is similar to AOP for skin sensitization, but with specific differences. The composition of these AOPs can provide insights into the prediction of differences in respiratory sensitivity from non-cutaneous sensitivity, an unclear regulatory requirement [25].

The AOP not only provides a mechanistic understanding of how the processes of skin and respiratory sensitization differ from each other, but also the need for further research to confirm the uncertainty of chemical respiratory sensitization [26]. Allergic hypersensitivity to the respiratory tract by chemicals is associated with rhinitis and asthma and remains an important occupational health problem. Recently, the concept of AOP has briefly described the major steps leading to health hazards, while drawing considerable attention from the toxicologist community as a basis for developing a framework and focus on future research [27].

Allergic contact dermatitis (ACD) is a hypersensitive immune response induced by small protein-reactive chemicals that can be identified and quantitatively assessed by local lymph node assays (LLNAs). Given the complexity of the ACD, a single alternative cannot replace LLNA, but integrated testing strategies (ITS) can be used to gather important information about molecular mechanisms related to skin sensitivity and to understand the molecular process of the ACD. A systematic review of *in vivo* studies has been carried out, and an ACD molecular map is prepared. Also, limited comparisons of toxic genomes in humans and mice have revealed similarities, although additional data are needed to identify subtle differences. For insight into the molecular mechanisms represented by various *in vitro* systems, comparative analysis with *in vitro* toxicogenomic data can be used to compare new molecular *in vitro* methods, in addition to mathematical predictability. In addition, new classifications by *in silico*, *in chemico*, and *in vitro* methods can be complemented by immobilizing AOP and ACD molecular maps, thus laying the foundation for developing test strategies that accurately reflect key events of skin sensitivity [28]. Many of these case studies have shown ways to inform AOP development in chemical hazard assessment [29]. AOP technology provides a means of organizing and sharing knowledge of the mechanisms of chemical toxicity, including quantitative structure–activity relationships

(QSAR) based on chemical and computational, *in vitro*, and integrated testing strategies, including toxicologically relevant readings. It comprises an explanatory method or integrated approaches to testing and assessment (IATA) that can be used as the basis for predictive techniques. The recently published OECD guideline provides guidance on what information is needed to identify and document AOP in terms of relevance and suitability [30].

Nanomaterials and AOPs

According to the OECD guidelines, many AOPs have been proposed for chemically induced side effects in humans and the environment, and in particular, the toxic effects of nanomaterials differ from the general mechanism, due to their inherent properties, making them difficult to compare directly. While there are many knowledge gaps in understanding nanomaterials toxicity, more and more studies are being published on toxicological properties. AOP can be used to reasonably combine mechanical knowledge related to toxicity by nanomaterials to fill data gaps and develop toxicological testing strategies, captured by AOPs selected for chemical-induced toxicity. Much of the mechanistic knowledge that has been gained can be used to provide nanomaterial toxicity prediction modeling and hazard assessments [31].

Due to the small size of the manufactured nanomaterials, there may be unknown health hazards, particularly in the working environment; the dust of certain manufactured nanomaterials affects the risk of inhalation and lung function. This fact requires rapid, cost-effective, and safe assays, such as multi-parametric high-throughput screening with cultured human lung cells. The predicted value of these *in vitro* tests is partially associated with mechanisms that contribute to their toxicity. Numerous adverse effects on fabricated nanomaterials, such as multi-walled carbon nanotubes due to changes in the dielectric, have been identified, one of which has recently been evaluated as carcinogenic. It is currently being used as a state-of-the-art strategy for analyzing genome effects from exposure to manufactured nanomaterials, focusing on lung disease [32]. Although a variety of engineered nanomaterials are used commercially with a variety of physicochemical properties with respect to *in vivo* toxicity effects, an integrated framework for human health risk assessment (HHRA) of ENMs is still not established. Two-year carcinogenicity studies, clinical chemistry, and histopathological endpoints in rodents have been considered the “gold standard” for detecting substance-induced toxicity in animal models, but genome-wide expression analysis and *in vivo* high-throughput testing (HTS) are increasing. In addition, these assays have shown that transcriptomics can be used as an effective mechanism-based method of

determining acceptable levels of exposure to nanomaterials in product development, when epidemiological studies are not possible.

Chemical carcinogenicity prediction and AOPs

Because some chemicals have multiple pathways of toxicity [33], a chemical risk assessment must include qualitative and quantitative information for each chemical involved [34]. Many international organizations have disseminated extensive information on chemical characterization, structure, toxicity, and risk assessment databases (e.g., PubChem, TOXNET and INCHEM). The US EPA generally defines and conducts a risk assessment of chemicals in four processes: risk identification, dose–response, exposure assessment, and risk analysis [35]. Key components of risk assessment include MoA analysis and WoE using *in vivo* and *in vitro* experiments, absorption, distribution, metabolism, and excretion (ADME) information, generated by quantitative structure–activity relationships (QSAR) and based on statistical models, such as physiology-based toxic kinetics modeling [36, 37].

With the exception of low-probability but acute industrial chemical accidents, the focus is on the chronic exposure to chemicals in everyday life, and humans may be exposed to chemicals through inhalation, ingestion, and skin contact [38]. For example, chronic exposure to air pollutants, such as workplace exposures, contaminated water, pesticide-exposed crops, pharmaceuticals, cosmetics, and other sources of exposure, can lead to chronic toxicity, which could be delayed or extended [39]. Long-term accumulation of side effects may lead to genotoxic or nongenetic damage by increasing genetic instability, which increases the likelihood of cancer becoming an end point [40–43]. Carcinogens can cause various cellular abnormalities, due to genetic or epigenetic changes [42].

AOP has a practical and robust weighting based on molecular, pathological, regulatory, and clinical knowledge, which facilitates the interpretation and integration of epidemiological studies in the hazard assessment process by describing biologically possible causes. The proposed AOP will present potential integration techniques for testing and evaluation to address the risks posed by chemicals in the future and will enable the development of biological instruments (e.g., Omics technology, stem cell culture, as well as experimental techniques, artificial tissues, etc.), using new techniques for understanding disease and toxicity that have traditionally been performed [44]. The goal of this new tool is to improve chemical risk assessment and reduce uncertainty. Recently, this concept has formalized the AOP for human health and ecological risk assessment and has been adopted as an OECD test guide.

Development of knowledge-based AOP

The implementation of AOP generates data and knowledge describing MIEs, intermediate effects, and toxic expressions (i.e., adverse outcomes), and AOP-related data can be viewed to facilitate the collection and retrieval of this information. Many research institutes have led and participated in creating flexible and standardized methods. Chemical specificity for intermediate effects is used in hazard assessment in all kinds of situations, since the OECD has adopted and developed a standard data format of OECD harmonized templates (OHT). Collecting historical data, the interim effects reported using OHT 201 were linked to each other to create an AOP, in accordance with the AOP technology framework [30, 45, 46].

Adverse outcome pathways describe generalizations and predictable biological motivations when certain biological pathways or processes are disturbed [13]. AOP represents existing knowledge linking two junctions, MIE and adverse outcome. For humidifier disinfectants, MIE is known to include ROS production, T cell reduction, and pro-inflammatory cytokine release from macrophages along the AOP. Possible AO may be causally related to major events (KE), interstitial fibrosis and pneumonia, asthma, allergic rhinitis or dermatitis, cerebrovascular and cardiovascular disease, diabetes, fetal death, premature bleeding, such as autoimmune diseases, liver and kidney toxicity, and cancer.

Epidemiological and toxicological studies using national health insurance data and AOP knowledge-based big data can verify the actual risk of AO. These new methods can be used to identify potential diseases when exposed to humidifier disinfectants. Therefore, next-generation tools, such as AOP and pathway-based toxicology, can be an appropriate way to clarify the toxicological effects of humidifier disinfectants. Previous reports have estimated that about two million people will be affected by a humidifier disinfectant, whether cured or not, and previous studies have shown specific findings focused on epilepsy fibrosis, compared to other diseases. AOP has been recommended as a new approach to understanding the overall impact of humidifier disinfectants, and epidemiological and toxicological studies using national health insurance data and AOP knowledge-based big data can identify the actual risk of toxic expression and apply this new method. This will provide a list of potential diseases when exposed to humidifier disinfectants [47]. In MIEs from humidifier disinfectants, according to a study by Song et al. [48], the administration of polyhexamethylene guanidine (PHMG) in the thymus causes elevation of pre-inflammatory cytokines and lung infiltration of immune cells. Reducing the total cell count and the ratio of CD4⁺/CD8⁺ cells, histopathological examination showed a significant decrease in the cortex and medulla, and mRNA levels with T cell development were also significantly reduced. It

was suggested that lung tissue exposure to PHMG not only causes lung inflammation and fibrosis, but also decreases cellular immunity [49, 50].

Discussion

Application of AOP in occupational health

AOP's conceptual framework, based on exposure and hazard data interpreted using risk assessment tools for product and environmental safety, can be used to understand side effects as a series of events or processes within biological systems and to improve current hazard assessment. While the precise definition of the MIE of a compound has not yet reached general acceptance, MIE is the initial interaction between a molecule and a biomolecule or biological system that may be causally related to the outcome through a route, the case study, and the issue of definition being addressed. Thus, the field can explore ways to use multiple processes of chemical knowledge to help further definition, classification, characterization, and hazard assessment and suggests the role of MIE research in the development of *in vitro* and *in silico* toxicology; studies are underway to identify and characterize MIE by a combination of chemical approaches [51].

The future application of AOP is in the possibility of predicting a decrease in lung function in humans exposed to potentially harmful inhalation toxicants; and in this context, the proposed AOP screens compounds that may pose a risk to humans during inhalation exposure. Cost-effective *in vitro* assays that can be further classified according to the degree of toxicity that may occur are being developed [52]. At the same time, biomarkers for developing preclinical endpoints that indicate the risk of death associated with a future disease or impaired lung function can also be derived from AOP [53–56]. In many studies, it is important to determine whether subsequent events, such as increased ROS levels compared to fresh air, include AOP-based EGFR activation and downstream signaling. AOP is a key combination of appropriate *in vitro* models and tests. It can provide a framework for the systematic assessment of events (KE). In addition, such AOP-based *in vitro* testing may ultimately be useful in knowing the dangers of chemicals and reducing their use [11, 57–61].

Adverse outcome pathways can play a role in integrating knowledge derived from a variety of sources, including experimental data, as well as evolution-based models. This will enable more efficient application of AOP knowledge of future extrapolation of species, as well as quantitative chemistry and site-specific hazard assessments [62]. Newly proposed AOPs can serve a variety of purposes, including the establishment of structure–activity relationships (SARs),

the development of new *in vitro* toxicity studies, and the refinement of priority strategies [12].

While genetic and epigenetic data usage for the prediction and identification of chemical hazards is growing, further research is needed to confirm MoA and AOP at a limited level, before the risk of dose–response modeling, exposure and/or risk assessment, and genetic/epigenetic variation is quantified. Evaluating gene–environment interactions is the basis from which genetic and epigenetic (epigenomic) data can be used for hazard and risk assessment. *In vivo* and *in vitro* data should be supplemented with human data collected from occupational health and molecular epidemiological studies. Research needs to be designed to provide as much information as possible about the relationship between genetic and epigenetic mutations and toxic pathways. In addition, attention should be paid to ethical, legal, social, and political impacts when genetic and epigenetic (epigenomic) data are used for occupational health issues. Ultimately, studies published before genetic and epigenetic data are used to assess occupational health, and the development of occupational exposure levels (OELs) should demonstrate that these changes affect occupational exposure and toxic expression [63]; the use of computational biology and toxicogenic pathways will increasingly focus on consistent physiological changes between similar groups of toxic substances [64, 65].

Developing non-animal testing techniques that utilize route-based machine information can provide more predictive tools for identifying potential risks, as well as more information on LC₅₀ or other *in vivo* observations. Extrapolating animal data to predict health effects may include physiological, anatomical, and metabolic differences between species (e.g., other airway dichotomy, cell type and composition, other biomodified enzymes and respiratory patterns, metabolic rate, physiological changes) [66]. It should also be sufficient to predict and manage potential adverse outcomes in humans [67–70].

Application for inhalation toxicity

The discovery of various AOPs after inhalation exposure opens the door to the development of *in vitro* and *in silico* technologies to assess AOP-related endpoints that can explain toxic expression at the organism and/or population level [13, 14]. These AOP tools provide more reliable information, assess target organ impacts, and help better understand how certain toxic chemicals affect humans (i.e., providing mechanistic insight values beyond what can be collected in the LC₅₀). This technology has become a promising field of research that has not yet been accepted by international regulatory bodies [71], and this review discusses the development of integrated approaches to testing and assessment (IATA) that can replace the use of animals

to assess the inhalation toxicity of chemicals. As more data and tools become available and understanding of the toxic mechanisms progresses, optimal technologies will continue to evolve. In addition to acute inhalation toxicity, many cellular systems and concepts can be applied to long-term repeated inhalation studies [72].

New approaches are needed to assess the outcomes of inhalation toxicants on workers' health, and these techniques will be based on the toxic mechanisms, understanding of dose measurement, *in silico* modeling, and *in vitro* testing. To accelerate the widespread implementation of these techniques, the development of AOP can help resolve data gaps by understanding models and mechanisms and can be used to investigate and optimize key events. Advances in the twenty-first-century life sciences are important to provide an unprecedented opportunity to gain a dynamic understanding of the causes and pathophysiology of disease and a concrete understanding of human beings, and to consider the ongoing trial and error of research, drug development, and clinical use [73]. Although new technologies can also be applied to environmental health and disease, these advances require new medical and drug development paradigms for maximum benefit. Human diseases should be used in systems toxicology for integrating and interpreting data on the cause of disease and pathophysiology, with studies focused on human-specific models for understanding AOPs at different biological levels, similar to those of toxicology [74, 75]. It is now time for an integrated discourse to identify and consider the many challenges and questions that need to be addressed.

This review summarizes current scientific advances on the mechanisms and assays that can be used to assess the inhalation toxicity of chemicals from the perspective of protecting workers' health. Although there are few QSAR models currently available for predicting inhalation toxicity, the applicability areas of QSAR (e.g., TopKAT and MultiCASE) are examined to determine whether a model can be optimized for occupational toxicity prediction. It is necessary to identify the differences between the advantages of existing models and to clarify which models can be used for specific applications. Also, new models can be developed using data collected from other sources, and the US EPA Center for computational toxicology (<https://comptox.epa.gov/dashboard/>) is a convenient platform to accommodate available models and their predictions [71]. It is important to carefully assess the variability associated with *in vivo* data that rely on developing new models to quantify the uncertainty associated with model prediction, and best practice protocols are being developed in *in silico* methods involving various endpoints [76]. There are also a number of AOPs involved in exposure, but additional AOPs need to develop specific toxic expressions that may occur with inhalation exposure. This will be useful for the ongoing construction of AOP on mechanisms of inhalation toxicity.

AOP development can be used for the likelihood of causing specific toxicity and to establish confidence in *in vitro* tests characterizing key events (KEs). For example, each researcher should also be encouraged to work with an AOP expert to bridge the gap between AOP development and knowledge, by providing an interactive virtual platform for AOP using an AOP-Wiki designed to create international consensus on the developed AOP. Many systems can be used for the evaluation of inhalable toxicants, but cell-based systems also need to be specific for their ability to metabolize compounds. In particular, three-dimensional (3D) tissue and lung-on-a-chip models are believed to represent human characteristics, but should be developed as a standardized test protocol to maintain consistency between laboratories.

Designing comprehensive assays for inhalation toxicity requires the use of AOPs, alternative or non-test methods, and *in vitro* assays. Experts with a variety of expertise (*in vitro*, *in vivo* inhalation toxicity, computational modeling, exposure science, etc.) should work together to design this approach. A key step in this process is proof-of-concept testing that focuses on specific chemicals, such as industrial and environmental toxicants, pesticides, tobacco, or pharmaceuticals, and is useful in describing key events in the pathogenesis for a particular AOP. The development and implementation of non-animal assays for inhalation toxicity testing and the acceptance of global regulations require cooperation between various stakeholders, and the International Science Consortium and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) have formed a working group. Researchers' participation is encouraged in these activities. The success in this area will develop platforms that can predict mortality and preventive effects from inhalation exposure, and these new techniques will lead to a better understanding of toxic mechanisms, without the use of animals, unleashing the potential of protecting workers' health for science of the twenty-first century.

Methods

This study examined the concept of AOP on workers' chemical exposure and prospects in occupational health. This review provides a summary of the current literature on AOP in occupational health, risk assessment of respiratory and nanomaterials, and prediction of carcinogenicity. Major Web sites were searched, specifically Google Scholar (<http://scholar.google.com>), ScienceDirect (www.sciencedirect.com), Scopus (www.scopus.com), NDSL (<http://www.ndsl.kr/index.do>), and PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>). The following key terms were used: "Adverse outcome pathway," "Toxicology," "Risk assessment," "Human exposure," "Worker," and "Occupational safety and health."

Among the literature searched through this process, the literature on toxic expression pathways (AOPs) was intended to utilize the most recent reports (after 2015), and the data on the research process and basic concepts that are presented for ease of explanation are not only from 2015, but also include some data from before 2015.

To accomplish the objective of this review, two categories of papers were selected: “AOP concept” and “Trends and applications of AOPs in occupational health.” Approximately 100 papers appeared in search engine results, with 75 papers meeting inclusion criteria for detailed analysis. Information from these 75 papers regarding measures for worker safety in the prospects for AOP in industry and occupational health has been summarized in this paper.

Conclusion

In order to fully understand adverse outcome pathway (AOP), it is necessary to consider the nature of genetic and epigenetic information. The assessment of the hazard and risk of industrial chemicals requires the results of *in vitro*, *in vivo*, and epidemiological studies on genetic and epigenetic factors and quantitative risk assessment studies. However, despite the great potential, little genetic or epigenetic (epigenomic) information has been used in the quantitative risk assessment of occupational and/or environmental exposures, and the number of studies containing this information will increase in the future.

Adverse outcome pathway is a conceptual tool that delineates the causal pathways of chemical toxicity. The goal of AOP is to accurately predict the toxicity of chemicals, by serving as an alternative to traditional chemical risk assessment. First introduced for ecological toxicity, AOP is now widely used in chemical assessments. The AOP, which is applied to inhalation toxicity especially carcinogenicity assessment, will be a tool to understand the effects of the use of chemicals in the workplace and the chronic exposure of the workers involved and make their predictions very useful. Based on this forecasting tool, it is expected to bring dramatic advances in occupational and environmental cancer prevention.

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Compliance with ethical standards

Conflict of interest Kyung-Taek Rim declares that he has no conflict of interest.

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