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The application progress of organoid technology in the toxicity assessment of environmental pollutants

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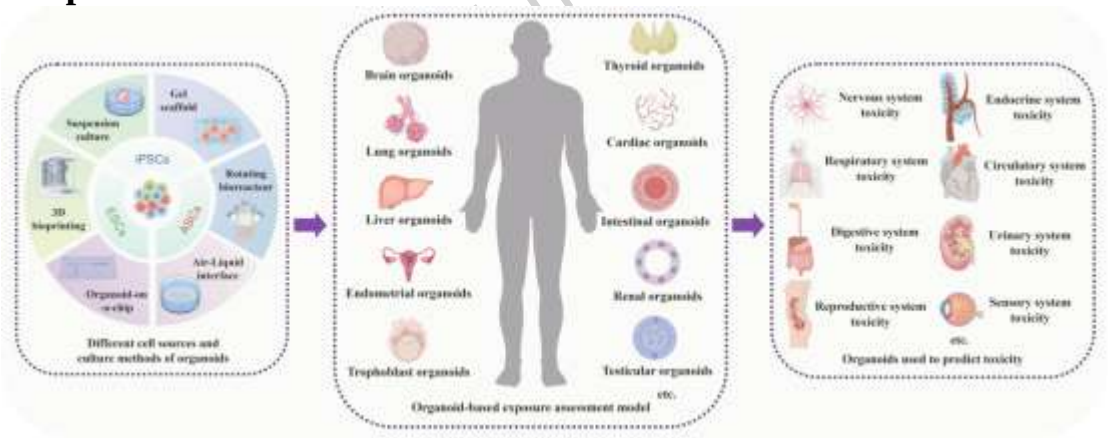
Abstract

Human production activities and daily life are the main sources of environmental pollutants, and their potential health impacts have attracted growing attention. Currently, the assessment of environmental pollutant toxicity depends heavily on animal models and some cell culture models. However, these approaches have inherent limitations, such as their inability to recapitulate the *in vivo* microenvironment and the presence of interspecies differences. Organoids not only recapitulate cell types and tissue structures of human organs, but also exhibit key physiological functions, rendering them ideal alternative models for assessing the health risks of environmental pollutants. In this review, we briefly introduce the development history of organoids as models for environmental pollutant toxicity assessment. Additionally, organoids are categorized by cell sources, and current mainstream organoid culture techniques are systematically summarized. Next, we highlight the application value of typical organoids including lung, brain, cardiac, intestine, liver and kidney organoids in environmental pollutant toxicity assessment. Finally, we thoroughly analyze the strengths and limitations of organoid models for such toxicity testing. Therefore, this review aims to advance organoid technology, and provide new insights into the toxicity assessment of environmental pollutants and the exploration of relevant toxic mechanisms.

Keywords: Environmental pollutant; Organoids; Toxicity test models; Risk assessment; *In vitro*

Graphical abstract



1 Introduction

Environmental pollutants spread and accumulate in soil, air and water, threatening ecological security and human health. Common types include microplastics (MPs), nanoplastics (NPs), endocrine-disrupting chemicals (EDCs), persistent organic pollutants (POPs), pesticides, air pollutants and heavy metals [1,2]. Prolonged or repeated human exposure can pose severe health hazards, including developmental abnormalities, carcinogenesis, reproductive damage and immune dysfunction [3]. Although existing toxicological studies are abundant, the molecular mechanisms of pollutant toxicity remain poorly understood [4]. Traditionally, toxicity assessment of environmental pollutants has relied primarily on two-dimensional (2D)

cell culture models and animal models. Conventional 2D cultures fail to recapitulate the complex in vivo microenvironment, while animal models have inherent interspecies differences. In addition, animal experiments are restricted by ethical issues, high costs and long cycles, which hinder large-scale application [5]. Therefore, novel physiologically relevant models are urgently needed for environmental toxicology research [6]. Recent advances in organoid, 3D bioprinting, gene editing and organ-on-a-chip technologies have broken through the drawbacks of traditional methods and improved the accuracy of toxicity prediction [7]. The U.S. Food and Drug Administration encourages the adoption of new approach methodologies to replace animal experiments. It endorses the application of organ-on-a-chip, organoids, AI-based toxicology models and other technologies in toxicological assessment, aiming to gradually reduce the use of animal testing and drive the shift toward a human biology-based research and development system. For regulatory review of data generated by new approach methodologies, the core validation principles are defined as clear application scope, human biological relevance, technical reliability and fit-for-purpose performance.

Organoids are 3D tissue-like constructs generated from stem cells, somatic cells, primary cells, or cells derived from tumor and normal tissues under defined culture conditions. By faithfully recapitulating human-specific tissue architectures, cellular crosstalk, and toxicological response patterns, organoids effectively avoid interspecies biases inherent in animal models. Therefore, they serve as a superior physiological platform over traditional 2D cultures and animal models to explore human toxic reactions and pollutant toxicity mechanisms [8]. Common organoid culture methods include suspension culture, air–liquid interface (ALI) culture, rotating bioreactor culture and gel scaffold culture. Advanced techniques such as 3D bioprinting and organoid-on-a-chip are also widely applied in this field [9]. With tissue-mimicking functions, organoids are ideal in vitro models for studying cell crosstalk, tissue development and pollutant toxicity, and help predict pollutant-induced health damage in humans. Currently, organoids are extensively used in disease modeling, drug screening, cancer therapy and regenerative medicine. They also show great promise for multi-organ toxicology research [10,11]. As an established research tool in toxicology, organoid technology presents great potential for evaluating the toxic risks of environmental pollutants and deserves further in-depth exploration [12,13].

With the rapid advancement of organoid research, several reviews on its application progress and research value in environmental pollutant toxicity assessment have been published in recent years. For instance, Yang et al. reviewed the research status of organoid models in the exposure assessment of emerging pollutants, but they failed to focus on the application value and core advantages of different types of organoids as toxicity testing models in the toxicity assessment of environmental pollutants [14]. Cong et al. merely elaborated on the application progress and prospects of organoids in assessing the human health risks posed by single-type environmental pollutants [15]. In addition, the research scope of some reviews is

relatively limited, which mainly focuses on the application progress of organoids constructed by a single culture technology in the toxicity assessment of environmental pollutants [16,17]. Against this background, the present review comprehensively elaborates the latest progress of organoid-based pollutant toxicity assessment. It first briefly introduces the development history of related models, and classifies organoids by cell sources alongside routine culture methods. Then it emphasizes the application potential of various organoids, with a special focus on recent findings and unique merits of lung, brain, intestinal, liver, kidney and heart organoids in toxicological studies. This paper also analyzes the strengths and inherent limitations of current pollutant exposure models. By synthesizing existing studies, we point out research gaps and future trends, and clarify the core role of human organoids in hazard assessment. This work is expected to provide references for developing new strategies in environmental toxicology.

2 Development of organoids as models for toxicity assessment of environmental pollutants

In recent years, organoid technology has developed rapidly with fruitful achievements (Figure 1). The relevant research dates back to 1907, when Wilson found that dissociated sponge cells could reaggregate and self-organize into organisms with normal physiological functions, laying a theoretical and experimental foundation for organoid self-assembly [18]. The term "organoid" was officially coined in 1954, marking the initial establishment of the conceptual framework in this field [19]. In 1981, mouse embryonic stem cells (ESCs) were successfully isolated for the first time. This breakthrough provided core seed cells for organoid research and also paved the way for the application of induced pluripotent stem cells (iPSCs) in organoid construction [20]. Advances in stem cell technology are the core driving force behind the development of organoids. In 1971, Friedenstein's team first isolated human mesenchymal stem cells (MSCs) from bone marrow [21]. In 1998, Thomson et al. successfully cultured human ESCs with typical biological characteristics [22]. In 2007, Takahashi et al. generated iPSCs via cellular reprogramming, representing a major breakthrough for stem cell and organoid research [23]. Currently, most non-tumor-derived human organoids are differentiated from pluripotent stem cells (PSCs) or iPSCs, which greatly facilitates their application in simulating organ physiological functions. In 2009, Clevers' team constructed intestinal organoids with crypt-villus structures using $Lgr5^{+}$ adult stem cells (ASCs) [24]. Since then, retinal, cerebral and other types of organoids have been developed, which can replicate tissue development and serve for disease research [25,26]. From 2014 onward, organ-specific organoids such as prostate, lung and mammary gland organoids were established successively, marking the gradual maturity of relevant preparation techniques [27]. In 2018, organoid technology was named the 2017 Method of the Year by Nature Methods.

Over the past decade, tissue- and stem cell-derived organoids have been widely adopted for the toxicological evaluation of environmental pollutants. The year 2013

marked the starting point of organoid-based toxicity testing for environmental contaminants, and this field has experienced rapid growth thereafter [28]. A large body of application-oriented studies has since emerged, focusing on mainstream pollutants including air pollutants, heavy metals, endocrine disruptors, MPs and related nanomaterials. In 2014, Astashkina et al. fabricated 3D proximal renal tubule organoids using medical-grade hyaluronic acid hydrogel to evaluate the toxic effects of nanoparticles. The results revealed that the reductions in cell viability and fluctuations in renal biomarkers induced by nanoparticles were consistent with the reference baseline obtained from rodent experiments. Different from traditional animal models, these human-derived organoids can recapitulate human-specific toxic responses, serving as a superior system for toxicity assessment [29]. Subsequently, organoids have been progressively integrated into the alternative model system for environmental pollutant toxicity testing, serving as a novel tool that more closely recapitulates physiological conditions for in vitro assessments in this domain. In 2015, Hofmann et al. used a co-culture system of organotypic lung tissues and immortalized alveolar macrophages to quantify inflammatory responses triggered by silica, a typical air pollutant, further proving the reliability of organoid platforms for toxicological analysis [30]. As research progressed, organoid models were applied to assess endocrine-disrupting chemicals. In 2016, Park et al. reported that 6-formylindolo[3,2-b]carbazole and 2,3,7,8-tetrachlorodibenzo-p-dioxin could inhibit the growth of mouse crypt- and $Lgr5^{+}$ cell-derived organoids [31]. Heavy metal toxicity has also become a major research direction. In 2018, Yin et al. combined brain organoids with organ-on-a-chip systems to explore neurological damage caused by cadmium exposure [32]. Alongside technical improvements, research has shifted from phenotypic observation to mechanistic exploration. In 2020, combined single-cell and spatial transcriptomics revealed that diesel particulate matter, a typical air pollutant, exerted adverse toxicogenomic effects on human brain organoids and hindered fetal brain development [33]. Bisphenol A and benzophenone-3, ubiquitous endocrine disruptors, were found to disrupt protein synthesis and transcriptional regulation during mammary gland organoid differentiation [34]. In 2022, Hua et al. observed that polystyrene microplastics caused developmental defects in forebrain cortical organoids in a size- and concentration-dependent fashion [35]. Reproductive and developmental toxicities are key research focuses in this field. In 2024, endometrial organoids were constructed to evaluate the reproductive damage caused by bisphenol A [36]. Since then, more sophisticated organoid models have been applied to identify the toxic hazards of environmental pollutants to specific target organs, including retinal organoids [37,38], thyroid organoids [39], and pancreatic organoids [40].

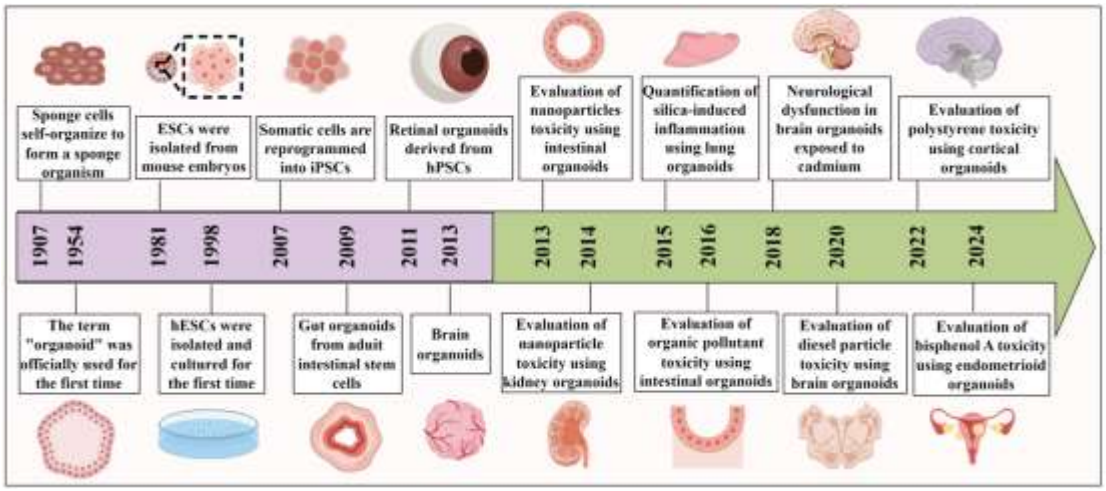


Figure 1. Development history of organoids. This figure outlines the key milestone studies and breakthroughs that have advanced the establishment of diverse organoid technology systems (denoted by purple arrows in the figure) and presents a timeline of organoids' evolution as a model for environmental pollutant toxicity testing (denoted by green arrows in the figure).

3. Classification and culture methods of organoids

3.1 Classification of organoids

The emergence of organoid technology relies on dual foundations: technological innovations in stem cell and developmental biology, as well as solid theoretical and experimental bases from classical developmental theories and cell isolation and reaggregation studies. A variety of classification systems have been established to categorize organoids based on different criteria, such as tissue origin, germ layer derivation, and cell source. Among these classification strategies, typing according to the source cell type is the most fundamental and widely adopted standard, because it essentially determines the differentiation potency, structural characteristics, functional properties, and applicable research scenarios of different organoid models (Figure 2) [41]. PSCs, consisting of ESCs and iPSCs, are characterized by superior pluripotent differentiation capacity. With precise in vitro directional induction and culture regulation, PSCs are capable of differentiating into organoids that simulate the structures and functions of almost all vital human organs, covering the brain, liver, kidney, lung and other key tissues. Importantly, PSC-derived organoids possess unique technical and ethical advantages: they support long-term unlimited in vitro expansion to meet the demands of repeated experiments and large-scale sample acquisition, and successfully avoid the ethical controversies inherent to human embryonic material usage. In addition, such organoids can faithfully recapitulate the complete genetic background of individual donors, rendering them ideal research models for exploring developmental toxicological mechanisms and analyzing personalized biological responses to environmental pollutants and exogenous stimuli [42]. Different from PSC-derived organoids, ASC-derived organoids are generated from tissue-resident stem cell populations distributed in mature somatic tissues, with typical representatives including $Lgr5^{+}$ intestinal crypt stem cells and mammary gland

stem cells. Such ASCs possess unique tissue-specific differentiation potency and only differentiate into cell types matching their original tissues, which endows them with stable tissue regeneration capacity under optimized in vitro three-dimensional culture conditions. These somatic stem cells can spontaneously self-assemble and differentiate into intact organoid structures that highly simulate the morphological characteristics and physiological functions of primary tissues. A mature and classical culture system is established for intestinal organoids: isolated mouse intestinal crypt $Lgr5^{+}$ stem cells can develop into standardized intestinal organoids with complete and mature crypt-villus structures in medium containing key regulatory factors (Wnt3a, R-spondin1, etc.), which stably retain the inherent absorption and secretory physiological functions of normal intestinal tissues [43]. Notably, the construction of different germ layer-derived organoids presents distinct cell dependence and technical specificity. Neuroectodermal organoids (brain organoids, optic cup organoids) and mesoderm-derived kidney organoids can only be successfully constructed through directed differentiation of PSCs due to their complex developmental characteristics. In comparison, surface ectoderm-derived glandular organoids, represented by salivary gland and mammary gland organoids, mainly depend on ASCs or isolated primary adult tissue cells for in vitro reconstruction. Differently, endodermal organoids including intestinal, hepatic and pulmonary organoids exhibit flexible cell source compatibility, which can be efficiently constructed via either PSC directed differentiation or ASC in vitro culture [44,45]. As another vital research model, tumor-derived organoids are established using clinical tumor biopsy tissues or surgically resected tumor specimens based on the self-assembly ability of endogenous cancer stem cells. This type of organoid model can faithfully preserve the core biological characteristics of primary tumors, including tumor heterogeneity, genetic mutation spectra and clinical drug sensitivity. Benefiting from the accurate simulation of tumor microenvironment and pathological features, tumor-derived organoids have become advanced in vitro platforms for anti-tumor drug screening and tumor mechanism research, and also provide new technical support for exploring the carcinogenic risk and molecular toxicological mechanisms of environmental pollutants [46].

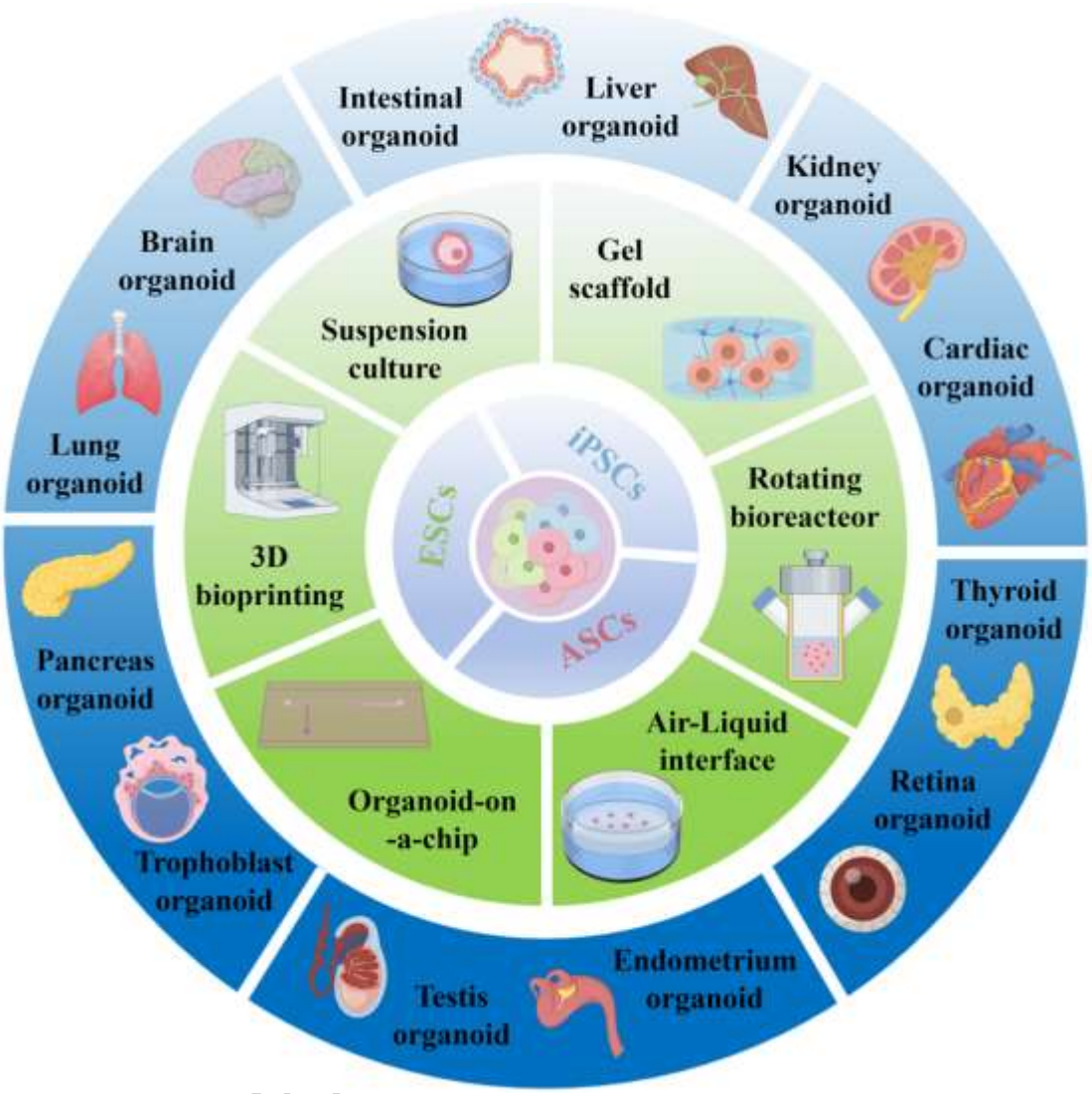


Figure 2. Different cell sources, culture methods and types of organoids.

3.2 Methods of organoid culture

Organoid culture is essentially a dynamic process that guides cells to self-assemble into functional structures by simulating the *in vivo* organ microenvironment. The academic community generally acknowledges four key elements for organoid formation and *in vitro* maintenance, including differentiation-competent stem cells, key factors regulating stem cell proliferation and lineage-specific differentiation, extracellular matrix (ECM) components for structural support, and nutrient-rich culture media to sustain cell survival and development [47]. Following years of exploration, researchers have successfully developed diverse organoid models, enabling the cultivation of organoids from distinct cellular sources. From a technical classification perspective, organoid cultivation techniques can be roughly grouped into four types, such as suspension culture, ALI culture, rotating bioreactor culture, and gel scaffold culture. In addition, innovative technologies like 3D bioprinting and organoid-on-a-chip have been widely adopted in organoid research. These technologies provide critical technical support for boosting organoid

growth efficiency, accelerating their maturation, and enhancing functional stability (Figure 2).

3.2.1 Organoid culture methods

Suspension culture is a scaffold-free 3D organoid fabrication method, where cells aggregate spontaneously, construct structures with self-secreted ECM and interact with each other to form intact organoids without adherent growth [48]. To avoid ECM hydrogels, Gong et al. developed a Matrigel-free suspension system for self-renewable hepatic ductal organoids; optimized culture yielded qualified organoids verified by viability, lineage and duct marker expression (Figure 3A) [49]. The scaffold-free suspension method may reform organoid culture for broader biomedical use. Baek's suspension system mimics in vivo conditions to study nanoparticle distribution/uptake and facilitates nanomaterial toxicity evaluation [50]. Suspension culture is easy and cost-saving without Matrigel, yet resultant organoids have loose structure, poor nutrient supply and immature function, limiting application and translation [51].

Rotary bioreactor is a typical dynamic 3D culture system. Continuous rotation builds fluidic and mechanical microenvironments to boost nutrient exchange and organoid maturation, supporting large-scale cultivation and translational development of organoids [52]. Przepiorski et al. used rotating bioreactor-based floating culture to induce embryoid body differentiation into kidney organoids, which formed functional nephron structures within 14 days (Figure 3B) [53]. This low-cost, scalable approach facilitates renal developmental and pathological research. Qian et al. developed a mini rotary bioreactor SpinΩ to produce region-specific human brain organoids from iPSCs that recapitulate human cerebral developmental features at multiple levels [54]. This compact system improves throughput and reproducibility for brain disease modeling and drug screening. Rotary bioreactors enable large-scale organoid culture for high-throughput toxicology tests of pollutant exposure, yet they suffer from high cost, complicated operation, harmful shear stress and incomplete organoid maturation; further optimization combined with co-culture or scaffold techniques is required [55].

ALI culture exposes one side of organoids to air and the other to medium, creating a polarized microenvironment to drive tissue-specific structure formation and functional maturation of organoids [56]. ALI culture suits organs with natural air-liquid interface including airway, skin and cornea. Wang et al. established an ALI-based RPMI 2650 nasal 3D model with respiratory epithelial and olfactory neuronal features including intact tight junctions, mucus secretion and neuronal differentiation, which supports combined respiratory and neurotoxicity assessment of airborne pollutants (Figure 3C) [57]. ALI culture promotes maturation of intestinal organoids for gut research and drug screening. Santos built an ALI culture from human small intestinal tissues containing native epithelium, mesenchyme and immune cells without exogenous reconstruction [58]. In addition, Tian et al. generated intact endometrial organoids via modified ECM combined with ALI co-culture of epithelial and stromal cells [59]. ALI culture is promising for organoid toxicology especially

PM2.5 pulmonary injury research, despite drawbacks in operation, scale-up and long-term culture sustainability [60].

3.2.2 Emerging organoid technologies

Gel scaffold culture uses biomimetic ECM gels to support organoid formation. tunable physical and biochemical properties of gels guide cell differentiation and self-assembly to form functional organ-specific structures [61,62]. Matrigel is widely used as gel scaffold for organoid disease and toxicology research. Ayabe team constructed patient-derived multi-structural biliary organoids in Matrigel, which recapitulated biliary epithelium and peribiliary glands and matured via TGF-β suppression [63]. In addition, Cao et al. established Matrigel-based forebrain organoids recapitulating multiple steps of early human cortical development. The model was verified with BPA for environmental neurotoxicity evaluation [64]. Matrigel's complex components, batch inconsistency and immunogenicity restrict its clinical translation [65]. Defined natural/synthetic hydrogels with adjustable structures and mechanics offer advantages; optimized biosignals, biophysical cues and crosslinking help mimic native microenvironment for reliable organoid studies [66]. Wang et al. 3D-bioprinted islet organoids using pancreatic ECM and methacrylated hyaluronic acid (MAHA) bioinks [67]. Decellularized scaffolds direct cell adhesion, migration and proliferation and are promising for tissue regeneration and organ substitution. Fabricating ECM-mimicking hydrogels for human iPSC culture is difficult. Against this backdrop, Roudaut's HA hydrogel supports iPSC aggregation and differentiation into functional liver organoids (Figure 3D) [68]. Low hydrogel solid content causes structural flaws, poor mechanics and over-swelling restricting organoid growth. Optimized crosslinking improves PEG-peptide hydrogel properties at low solid content to support mouse and human intestinal organoids with crypt formation comparable to Matrigel, advancing Matrigel-free organoid culture translation [69]. By virtue of their precise regulation of the microenvironment, hydrogels can flexibly fulfill the requirements for physical support, biosignals, and dynamic metabolic needs essential to organoid growth.

Organs-on-chips combine microfluidics and organoid culture to build biomimetic dynamic microenvironments, overcoming static culture drawbacks and enabling more physiologically relevant in vivo-like organoid modeling [70]. Traditional models lack perfusion, expandability and complex microenvironment compatibility, limiting translational application. Therefore, Maiullari et al. developed a microfluidic small vascular environment bioreactor (sVEB) chip to mimic perivascular niche for breast cancer research; co-culture of endothelial cells, breast cancer organoids and modified T cells recapitulates angiogenesis and tumor-immune crosstalk for mechanistic and therapeutic studies (Figure 3E) [71]. Organoids fail to fully recapitulate organ physiology owing to missing cell subtypes like neurons. Here, Fredrikson's organ-chip generates stable mature neural networks and co-cultures organoids to resolve asynchronous developmental signaling [72]. Various organs-on-chips for

major organs are under global development, yet complicated fabrication and high cost hinder their extensive application [73].

3D bioprinting is an interdisciplinary approach depositing cell-containing bioinks layer-by-layer to fabricate structurally intact and functional tissue/organ constructs [74]. 3D bioprinting surpasses conventional cell-seeding-based tissue engineering with precise structural control, homogeneous cell distribution and personalized manufacturing feasibility [75]. 3D-bioprinted organoids support developmental and toxicology research. Photo-crosslinking bioprinting generates testicular organoids resembling native testis in structure, cell arrangement and gene expression [76]. Additionally, Yao's team bioprinted patient-derived and healthy iPSC-based liver organoids mimicking hepatic space of Disse to explore MP-triggered hepatotoxicity, verifying the critical role of fibrosis-related genes (Figure 3F) [77]. Therefore, 3D-bioprinted biomimetic organ models mimic human physiology for precise assessment of drug efficacy and organ toxicities, minimizing interspecies biases of animal trials for drug and toxicology studies.

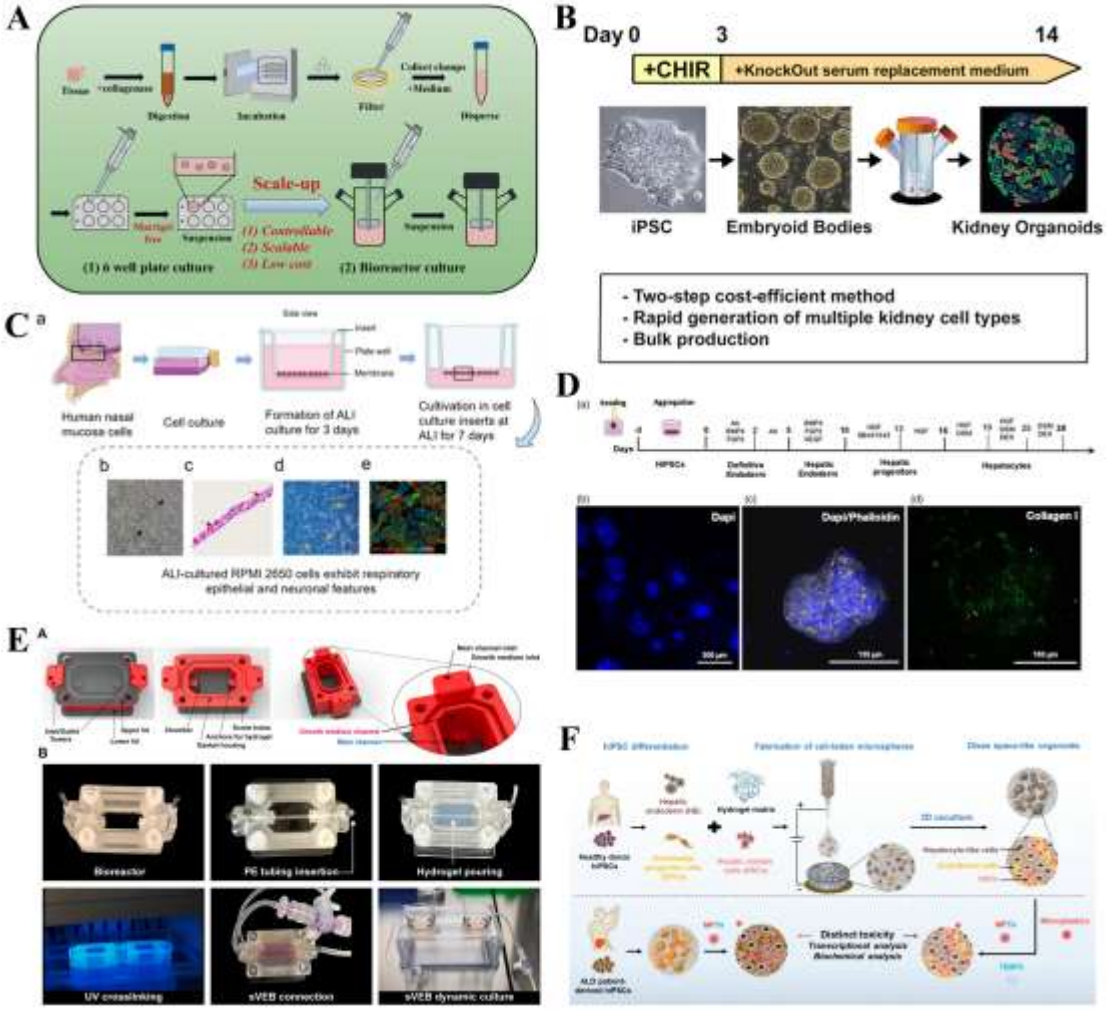


Figure 3. Methods of organoid culture. (A) A novel matrigel-free suspension culture enhanced the generation of high-quality human liver ductal organoids [49]. (B) Kidney organoids can be efficiently generated from human iPSCs using bioreactor technology [53]. (C) A 3D model of

human nasal RPMI 2650 cells was successfully established based on the ALI culture technology [57]. (D) Protocol for differentiating human iPSCs into liver organoids using Biomimesys® HA [68]. (E) A novel sVEB has been developed based on organ-on-a-chip technology and microfluidic technology [71]. (F) Distinct toxicity of MPs/TBBPA co-exposure to bioprinted liver organoids from hiPSCs of healthy and patient donors [77].

4 The application of organoids as a model for assessing the toxicity of environmental pollutants

Organoids have been widely used as toxicity testing models in toxicology, with particular significant value demonstrated in drug toxicity assessment. However, there remains a relative scarcity of review studies focusing on their application in environmental pollutant risk assessment. Therefore, this review takes lung, brain, heart, intestinal, liver and kidney organoids as the core research subjects. On the one hand, these organs are the major target organs most vulnerable to environmental pollutants after entering the human body. On the other hand, abundant achievements have been made in toxicological research on pollutants using the above organoids, and their culture and detection technical systems are well established (Table 1).

Table 1 Application of organoids technology in toxicity testing of environmental pollutants

Organoids	Sources	Culture methods	Pollutants	Dose	Toxicological effects	Refs.
Lung	Lung epithelial progenitors	Matrigel	Diesel exhaust particles	0, 50, 100 and 200 μg/mL	The number and average diameter of airway and alveolar organoids have significantly decreased	[79]
	Normal tissues of the distal lung airways	Matrigel	$PM_{2.5}$	50 μg/mL	Low-level $PM_{2.5}$ induced early lung injury by regulating circ_0092363	[80]
	ASCs	Matrigel	1-NP	1, 10 and 50 μM	Massive production of ROS and abnormal lipid metabolism	[81]
	ESCs	Matrigel	$PM_{2.5}$	0, 12.5 and 25 μg/mL	Inhibition of the proliferative activity and decreased expression levels of specific markers (NKX2.1, SOX2, SOX9)	[83]
	PSCs	Suspension culture	Diesel $PM_{2.5}$	0, 50, 100 and 200 μg/mL	Potential alveolar developmental toxicity and increased susceptibility to SARS-CoV-2 of diesel $PM_{2.5}$	[84]
	Primary human bronchial epithelial cells	Matrigel	Tire wear particles	0, 50 and 100 μg/mL	TWPs induced significant cell apoptosis and oxidative stress	[85]
	ESCs	Matrigel	Particulate matter	250 μg/mL	Particulate matter exacerbated cellular damage by increasing stress granule formation	[86]
	ESCs	Matrigel	PHMG-p	2.5 μg/ml	PHMG-p induced severe lung toxicity under stress	[87]

					conditions	
	ESCs	Suspension culture	$CdCl_2$	1, 3, or 6 μM	Cadmium exposure induced partial arrest in the development of brain organoids	[89]
	ESCs	Suspension culture	$Al(OH)_3$	0, 5, 25 μg/ml	Exposure to aluminum hydroxides inhibited the differentiation of brain organoids and significantly altered their transcriptomic profiles	[90]
	ESCs	Suspension culture	PFAS	10 ng/ml PFOS and PFOA, and 1 ng/ml PFHxS	PFAS exposure induced Alzheimer's disease-like neurotoxicity in cerebral organoids	[91]
Cerebral	iPSCs	3D bioprinting	DPM	30 mg	DPM interfered with the neural development process and increased the potential risk of autism spectrum disorder	[77]
	Mice and human iPSCs	Suspension culture	PP-NPs	10, 25, and 50 μg/mL	Human cerebral organoids exposed to PP-NPs exhibit reduced growth and neuronal differentiation, with significant downregulation of key neuronal markers such as TUJ1, MAP2, and PAX6.	[92]
	iPSCs	Suspension culture	MPs	2.5 mg/mL	Exposure to 50-nanometer MPs significantly reduced the viability of organoids and mediated neurotoxic effects by activating the kynurenine pathway	[93]
	iPSCs	Suspension culture	6PPD	0.1, 1, 10, and 100 μM	6PPD induces multiple cardiac injuries including apoptosis, electrophysiological disorders, DNA damage and endoplasmic reticulum stress in a dose-dependent manner.	[97]
cardiac	iPSCs	Organs-on-chips	PS-NPs	0, 30, 60, and 120 μg/mL	PS-NPs impair cardiac structure and function in a dose- and time-dependent manner.	[98]
	iPSCs	Matrigel	TCC	1, 2, 5 μmol/L	TCC induces myocardial hypertrophy and metabolic reprogramming in human cardiac organoids.	[99]
	Small intestinal tissue	Matrigel	$CdCl_2$	30 μM	Cadmium ingestion exacerbated *Salmonella* infection, with a loss of goblet cells through activation of notch signaling pathways in the intestine organoids	[101]
Intestinal	Intestinal epithelial cells	Scaffold culture	MPs	50 mg/L	Exposure to MPs impaired the ZO-1, thereby damaging the integrity of the cellular barrier and resulting in a significant decrease in TEER values	[102]
	ISCs	Matrigel	TCS and TCC	10 μM	Exposure to TCS and TCC significantly impaired the self-renewal capacity and differentiation process of ISCs	[103]
	Mouse pelleted	Matrigel	PSMP@B(a)P	60 μg/mL	PSMP@B(a)P promoted colonic barrier injury via oxidative stress-mediated notch signaling	[104]

	crypts					
	Mouse liver tissue	Scaffold culture	PFAS	100 mM	Long-chain PFAs induced abnormal cell morphology and impairment of ALT/GDH enzymes, whereas no changes in acute toxicity-related endpoint indicators or cell apoptosis were detected in response to short-chain PFAs	[108]
Liver	Mouse liver tissue	Matrigel	6-PPDQ	0, 40, 80, and 160 μg/L	Exposure to 6-PPDQ can significantly activate signaling pathways related to DNA replication and repair, carcinogenesis, and inflammatory responses, thereby inducing hepatotoxicity	[109]
	ESCs	Matrigel	HFRs	10 nM	HFRs disrupted hESC hepatic differentiation and promoted the proliferation of differentiated cells partly via up-regulation of the FGF10 signaling pathway	[110]
	Duct cells	Matrigel	TBPH	5 and 50 μM	TBPH promoted the progression of non-alcoholic steatohepatitis by disrupting endoplasmic reticulum-mitochondria contacts	[111]
	iPSCs	Matrigel	PS-MPs	0, 1.25, 2.5, 5, 10, 20 μg/mL	PS-MPs induced mitochondrial oxidative stress, activated the Bcl-2 protein family, and triggered cell apoptosis via the Bcl-2/Bax/caspase-9/caspase-3 signaling pathway	[113]
	iPSCs	Matrigel	PS-MPs	0, 0.625, 1.25, 2.5, 5, 10, and 20 μg/mL	PS-MPs reduced the size of kidney organoids by decreasing cell proliferation and increasing cell apoptosis	[114]
Kidney	Kidney proximal tubule epithelial cell	Scaffold culture	Gold NPs	3.5 and 56.6 μg/mL	Exposure to NPs induced the production of *in vivo*-relevant renal biomarkers and led to specific changes in cell viability	[29]
	iPSCs	Matrigel	PS-MPs	2.5 μg/mL	PS-MPs induced nephrotoxicity through DDIT4-mediated autophagy and apoptosis	[115]
Thyroid	ESCs	Matrigel	BAP	10 μM	BAP alone upregulated genes involved in cell cycle regulation, response to reactive oxygen species, and cell apoptosis	[116]
Endometrial	Endometrial glands	Matrigel	BPA	0.1, 0.5 μM	BPA induced structural and molecular damage in organoids, affecting cytoskeletal proteins, the Wnt/β-catenin signaling pathway, and epithelial/mesenchymal markers	[36]
Retinal	ESCs	Matrigel	Pesticides, flame retardants, PPCPs, and other	10, 100, and 1000 nM	Exposure to pollutants caused varying degrees of cell detachment	[37]

			typical pollutants			
Testicular	Rat testicular cells	Matrigel	MEHP and cadmium chloride	MEHP (0.5, 1, 1.5 μM); cadmium chloride (0.01, 0.05, 0.25, 1.25 μM)	After exposure to MEHP and cadmium chloride, organoids exhibited loss of the tight junction protein Claudin 11 and changes in the transcriptional levels of somatic cell markers	[117]
Trophoblast	Villi in first trimester placental tissue	Scaffold culture	EHDPP	100, 1000 or 10,000 nM	EHDPP disrupted placental formation in trophoblast organoids	[118]

4.1 Pulmonary toxicity

The lung is the core respiratory organ responsible for gas exchange, and it also mediates physiological stress responses to drugs, chemicals and environmental pollutants. Inhalation is a major route for human exposure to environmental pollutants. Over the past decade, various in vitro lung organoid models have been developed and widely applied to explore pollutant-induced pulmonary toxicity [78]. Lung organoids co-cultured with fibroblasts and alveolar epithelial progenitors have been used to investigate the toxicity of diesel exhaust particles (DEP). Results demonstrated that DEP disrupts alveolar epithelial signaling niches, thereby suppressing the growth and maturation of lung organoids [79]. Human PSC-derived lung organoids, which closely simulate native tissue structures and functions, serve as reliable models for airway toxicology research. Using human ESC-derived lung bud tip organoids, Xu et al. found that $PM_{2.5}$ inhibits cell proliferation and dysregulates lung development-related genes. They also identified circ_0092363 as a potential biomarker for early $PM_{2.5}$-induced lung injury [80]. Additionally, airway epithelial organoids derived from ASCs were adopted to evaluate 1-nitropyrene (1-NP) toxicity. 1-NP exposure triggers excessive reactive oxygen species (ROS) production and disturbs lipid and phospholipid metabolism in organoids [81]. Epidemiological studies have confirmed that heavy metals, POPs and air pollutants impair lung development. Superior to traditional 2D models, lung organoids faithfully recapitulate early organogenesis, making them ideal platforms to investigate pollutant-induced developmental pulmonary toxicity[82]. Wang et al. utilized human ESC-derived lung bud tip progenitor organoids (LPOs) to simulate fetal lung development. Their data revealed that $PM_{2.5}$ suppresses cell proliferation and alters the expression of lung progenitor markers (SOX9, SOX2, NKX2.1) [83]. Kim et al. further reported that diesel fine particulate matter (dPM2.5) interferes with the differentiation of PSC-derived alveolar epithelial cells and 3D alveolar organoids, accompanied by elevated NADPH oxidase expression and inflammatory reactions [84]. MPs have become a global public health concern. MPs ranging from 12 to 2475 μm can enter the respiratory tract via inhalation and accumulate in lung tissues. Jiang et al. used airway organoids to assess the toxicity of tire wear particles (TWPs), a typical MP derivative. TWPs

dose-dependently inhibit organoid growth, induce cell apoptosis and provoke intense oxidative stress (Figure 5A) [85]. Moreover, particulate matter aggravates cellular damage in respiratory syncytial virus (RSV)-infected lung organoids by promoting stress granule formation [86]. Similarly, polyhexamethylene guanidine phosphate (PHMG-P) elevates stress granule levels in RSV-infected 3D lung organoids, which exacerbates lung tissue injury caused by viral infection [87].

4.2 Neurotoxicity

Brain organoids can faithfully recapitulate the microenvironment of early human brain development and form functional neuronal networks and synapses. As a sensitive in vitro platform for neurotoxicity evaluation, they reduce animal reliance and enable high-throughput screening, facilitating mechanistic research on pollutant-induced neural damage [88]. The developing fetal brain is highly vulnerable to environmental pollutants, which may cause developmental defects and raise the risk of neurological disorders. It is therefore critical to clarify the neurotoxic effects and molecular mechanisms of pollutants for public neurological health protection. Using human ESC-derived brain organoids, Gao et al. demonstrated that Cadmium exposure causes severe damage and disordered patterns in neural development. This process is closely associated with the depletion of neural progenitor cells, impaired organoid integrity and ectopic compensatory cell proliferation [89]. In addition, Aluminum hydroxide exposure was found to reduce neuron numbers, disturb synaptogenesis and neuronal migration, and alter the transcriptome of brain organoids (Figure 5B) [90]. Brain organoids are also applied to explore pollutant-related neurological disease risks. Perfluorohexane sulfonate (PFHx), perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) induce Alzheimer's disease-like neuropathological changes and disrupt lipid metabolism, ultimately leading to Alzheimer's disease-like lesions in brain organoids [91]. Additionally, DPM impaired cellular respiration and mitochondrial function, potentially interfering with neurodevelopment and increasing the risk of autism spectrum disorder [77]. MPs can adsorb environmental toxins, yet research on their neurotoxicity and accumulation in the brain remains limited. Human iPSC-derived brain organoids help address this research gap. During the early developmental stage of human brain organoids, polypropylene nanoparticles suppressed the growth and neuronal differentiation of developing brain organoids, accompanied by downregulation of key neuronal markers including PAX6, MAP2 and TUJ1 [92]. Park et al. reported that 50 nm MPs decreased organoid viability and activated the kynurenine pathway, which elevated neurotoxic quinolinic acid levels [93]. However, A major limitation of conventional brain organoids is the lack of a native blood-brain barrier (BBB), which makes it impossible to simulate the physiological process of toxicants crossing the barrier. To solve this problem, Dao et al. constructed a miniature human brain model with intact functional BBB using neural stem cells [94]. This advanced model provides a powerful tool for studying BBB function and accurately assessing the neurotoxicity of environmental pollutants and drugs.

4.3 Cardiotoxicity

Human PSC-derived cardiac organoids are powerful tools to evaluate pollutant cardiotoxicity. These 3D multicellular structures faithfully reproduce human cardiac physiology and electrophysiology, supporting quantitative detection of pollutant-triggered heart injury [95,96]. Validated as robust in vitro platforms, human iPSC-derived cardiac organoids show that the tire additive 6PPD causes dose-dependent cardiomyocyte apoptosis and electrophysiological disorders. Cardiac dysfunction emerges even at non-cytotoxic low doses, supplying humanized evidence for 6PPD cardiovascular risk evaluation (Figure 5C) [97]. However, traditional cardiomyocyte cell lines cannot mimic native cardiac biological traits. To overcome this drawback, Zhang et al. constructed a cardiac organoid-on-a-chip system for polystyrene nanoparticles (PS-NPs) toxicity testing. PS-NPs impair cardiac structure and function in a dose- and time-dependent way, and low concentrations exacerbate hypoxia plus norepinephrine-mediated myocardial damage [98]. In addition, with transcriptomic and proteomic analyses, cardiac organoids resolve molecular toxic mechanisms. Yang et al. confirmed that TCC alters endothelial arginine metabolism and breaks NO homeostasis in cardiac organoids. Rising TCC doses induce ROS accumulation and iNOS overactivation, triggering endothelial nitrosative stress, inflammation and dysfunction, which ultimately drive myocardial hypertrophy [99]. Although insufficient vascularization and lack of immune microenvironment restrict current applications, optimized culture and chip technologies will render cardiac organoids a core in vitro model for pollutant cardiovascular toxicology research.

4.4 Enterotoxicity

Oral intake is a major route for human exposure to environmental pollutants, and the intestine serves as a primary target organ for their toxicity. Conventional in vitro gastrointestinal models cannot fully recapitulate the intestinal microenvironment, while intestinal organoids overcome this limitation and serve as superior platforms for evaluating pollutant penetration, intestinal barrier damage and related molecular mechanisms [100]. Studies have shown that Cadmium exposure induces excessive ROS production in intestinal organoids and further activates the Notch pathway. This pathway dysregulation reduces goblet cell numbers and mucin secretion, weakening intestinal mucosal defense and increasing susceptibility to Salmonella typhimurium infection [101]. The intestinal organoids with complex structures can accurately reflect the effects of environmental pollutants on the intestinal barrier and the underlying toxic mechanisms. NPs can trigger ROS accumulation, mediate intestinal epithelial cell apoptosis and aggravate intestinal barrier damage [102]. In addition, triclosan (TCS) and triclocarban (TCC) inhibit the Wnt signaling pathway, impairing the self-renewal and differentiation of intestinal stem cells (Figure 5D) [103]. Polystyrene microplastics loaded with benzo[a]pyrene (PSMP@B(a)P) trigger oxidative stress and activate the Notch pathway, eventually causing colonic barrier injury [104]. Beyond toxicological assessment, intestinal organoids also show great potential in developing and screening radioprotective agents. They can be used to test

protective efficacy and optimize relevant protocols, so as to reduce occupational radiation hazards [105].

4.5 Hepatotoxicity

Liver organoids are generally derived from human iPSCs or ASCs. Their multicellular, biomimetic structures faithfully recapitulate hepatic metabolism and toxic responses, serving as superior alternatives to traditional 2D cell and animal models [106]. Liver organoids constructed via co-culture of hepatocyte-like cells, vascular endothelial cells and umbilical cord mesenchymal stem cells develop intact $CD31^+$ vascular networks and $CK19^+$ bile duct structures, with improved albumin synthesis, glycogen storage, drug transport and xenobiotic metabolism [107]. Liver organoids are powerful tools for assessing the hepatotoxicity, genotoxicity, teratogenicity and carcinogenicity of environmental pollutants. Studies on perfluoroalkyl substances (PFAs) revealed distinct toxic effects by carbon chain length: long-chain PFAs altered cell morphology and suppressed alanine transaminase and glutamate dehydrogenase activities, while short-chain PFAs caused no obvious acute toxicity or cell apoptosis [108]. With high CYP450 activity and stable gene expression, liver organoids are well suited for toxicological research. Ge et al. adopted a stable liver organoid model to assess the hepatotoxicity of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6-PPDQ). Transcriptomic analysis indicated significant activation of pathways related to DNA repair, carcinogenesis and inflammation (Figure 5E) [109]. This model also facilitates in-depth exploration of pollutant-induced molecular changes. Using human ESC-derived liver organoids, Yang et al. found that halogenated flame retardants (HFRs) accelerate hepatoblast proliferation via the FGF10 signaling pathway [110]. Zhou et al. further reported that bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH) downregulates mitofusin 2 (MFN2). The decreased MFN2 disrupts endoplasmic reticulum-mitochondrial contacts and phospholipid transport, ultimately leading to mitochondrial dysfunction and progression of non-alcoholic steatohepatitis (NASH) [111].

4.6 Renal toxicity

The *in vitro* self-assembled kidney organoid models provide a novel research perspective for the development of kidney microphysiological platforms that can more reliably simulate the *in vivo* environment, and are anticipated to play a key role in the efficient screening of environmental pollutant-induced renal toxicity. Kidney organoids, as a novel 3D *in vitro* model, can not only precisely simulate the physiological development process of the kidney, but also serve as an ideal experimental model for exploring the mechanism of early kidney morphogenesis [112]. For example, polystyrene microplastics (PS-MPs) disrupted the development of kidney organoids through oxidative stress and the Bcl-2/Bax/caspase pathway [113]. Additionally, Xie et al. systematically explored the potential impacts of MPs on the early nephrogenesis process using kidney organoids derived from human iPSCs. The study not only uncovered the toxic effects of MPs on the structural integrity of renal

tubules and the progression of kidney development, but also supplemented critical theoretical basis for research on kidney developmental toxicity induced by environmental MPs (Figure 5F) [114]. Kidney organoids can assess the health risks of environmental pollutants by simulating biological effects under different pollutants and exposure levels. Astashkina et al. assessed the renal toxicity of NPs and gold NPs by 3D kidney organoids constructed from mouse proximal tubules suspended in a hydrogel based on biomedical-grade HA [29]. In addition, after exposing kidney organoids to PS-MPs across different concentration ranges for 24 hours, it was found that the overall size of the organoids was significantly reduced. Meanwhile, the expression levels of nephron-specific markers were substantially decreased, and the normal formation processes of both proximal and distal tubules were significantly impaired. These changes intuitively illustrate the renal toxicity of PS-MPs [115].

4.7 Application of organoids in other toxicity studies

Beyond the aforementioned organ types, a range of other organoid types have also been successfully constructed and applied to the toxicity assessment of environmental pollutants. Endocrine and reproductive organoids have emerged as precise and efficient in vitro models for exploring the toxic mechanisms of environmental EDC. In thyroid toxicity studies, Nazzari et al. adopted ESC-derived thyroid follicles to evaluate EDC toxicity. Both single and combined exposure to male mixed hormones and B(a)P significantly upregulated genes involved in lipid transport and metabolism [116]. To address the technical limitations and ethical issues of traditional animal assays, reproductive organoid models have been increasingly applied to clarify pollutant-induced reproductive damage. Abady et al., for example, utilized endometrial organoids to characterize the reproductive toxicity of BPA. BPA exposure induced structural and molecular disorders in endometrial organoids, altered cytoskeletal protein expression, disturbed Wnt/β-catenin signaling homeostasis, and remodeled the expression profile of epithelial-mesenchymal transition markers [36]. In testicular toxicological evaluation, Sakib et al. established testicular organoids using a microwell culture system. Exposure to mono(2-ethylhexyl) phthalate and cadmium chloride resulted in the loss of tight junction protein 11 and substantial transcriptional dysregulation of somatic cell markers [117]. In addition to mechanistic toxicological analysis, organoid platforms support high-throughput screening of environmental toxicants. Xu et al. developed a human trophoblast organoid-based immunofluorescence screening system to screen organophosphorus flame retardants with developmental toxicity. Three aryl organophosphate flame retardants markedly inhibited trophoblast organoid proliferation. Mechanistically, 2-ethylhexyl-diphenyl phosphate (EHDPP) disrupts insulin-like growth factor 1 receptor signaling, suppresses cellular aerobic respiration, and ultimately impairs trophoblast development and proliferation [118]. However, current research on the toxic mechanisms of environmental pollutants remains insufficient, particularly regarding the processes of distribution, metabolism, excretion, and damage after organisms ingest environmental pollutants. The establishment of relevant organoid models is

anticipated to offer support for a more thorough understanding of research on the toxic mechanisms of environmental pollutants *in vivo*.

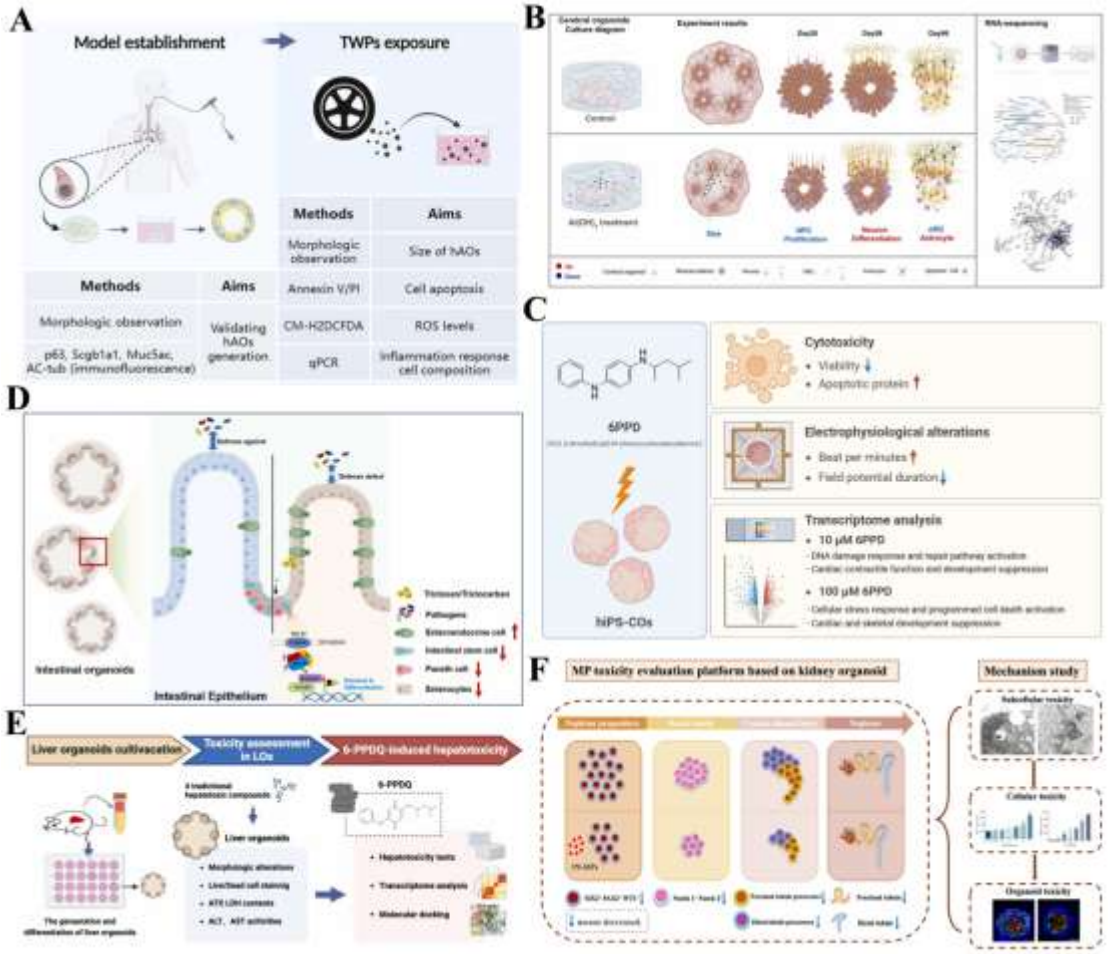


Figure 5. The application of organoids as a model for assessing the toxicity of environmental pollutants. (A) The airway toxicological effects of TWPs were evaluated based on the human airway organoid [85]. (B) Exposure to aluminum hydroxide induced neurodevelopmental impairment in brain organoids derived from human ESCs [90]. (C) 6PPD exerts multiple cardiotoxic effects on hiPSC-derived cardiac organoids, including decreased cell viability, activated apoptosis, electrophysiological dysfunction and transcriptomic molecular alterations [97]. (D) TCS/TCC impaired the self-renewal capacity and differentiation process of intestinal stem cells by inhibiting the activity of the Wnt signaling pathway [103]. (E) Liver organoids uncovered tire-derived 6-PPDQ-induced hepatotoxicity [109]. (F) The potential effects of MPs on the early renal development process were investigated using a kidney organoid derived from human iPSCs [114].

5 Advantages and challenges of organoids in toxicity testing of environmental pollutants

The application of organoids in pollutant exposure assessment is a frontier field bridging environmental toxicology and in vitro model research. Related technological advances have improved the risk assessment system for environmental pollutants and overcome the drawbacks of conventional evaluation methods. Featuring prominent biomimetic superiority to recapitulate in vivo physiological microenvironments,

organoids are promising alternatives to animal models and traditional 2D cell models. Toxicological data derived from organoid assays show better consistency with in vivo animal measurements compared with standard 2D cell culture, attracting extensive academic attention. Animal experiments serve as a classic approach for the toxicity evaluation of environmental pollutants. Nevertheless, this method consumes abundant laboratory animals with long experimental cycles and high costs. In addition, discrepancies in species and exposure routes lead to physiological differences between humans and experimental animals, making it difficult to directly extrapolate animal experimental results to humans and resulting in evident limitations [119]. As a novel in vitro model, organoids consist of multiple cell types and can mimic the structural and physiological functions of human organs. For instance, cardiac and intestinal organoids recapitulate myocardial contraction and intestinal crypt-villus architecture, respectively. They can accurately reflect organ toxicity induced by pollutants and overcome the limitations of traditional 2D cell culture and animal models[120]. Zhang et al. fabricated cardiac organoid-on-a-chip by combining cardiac organoid and organ-on-a-chip technologies. Equipped with multiple cardiomyocyte-specific cell types, this chip can mimic cardiac mechanical signal transduction and physiological myocardial contraction, providing a reliable in vitro research model for evaluating nanoparticle-induced cardiac toxicity [121]. Compared with animal experiments, toxicity tests based on organoids feature shorter cycles and higher efficiency, reduce the consumption of laboratory animals and experimental costs, and comply with the internationally recognized 3R ethical principles for animal research [122]. In addition, combined with transcriptome sequencing and gene editing technologies, organoids can be used to dissect the toxicological mechanisms of pollutants. Existing studies have verified that nanoparticle exposure triggers mitochondrial oxidative stress, activates the P38/Erk MAPK pathway and blocks autophagic flux in cardiac organoids, further inhibiting the pluripotency of human embryonic stem cells, which provides experimental evidence for clarifying the underlying mechanisms of nanoparticle cardiotoxicity [123]. Currently, the U.S. Environmental Protection Agency and the EU Registration, Evaluation, Authorization and Restriction of Chemicals Regulation have formally incorporated organoids into its regulatory assessment system and further promoted the adoption of non-animal testing to replace animal experiments. It emphasizes that organoid-derived data can serve as part of weight-of-evidence evaluation. Particularly in chemical safety assessment, such data may substitute for or complement conventional animal experiment data to assess risks including toxicity and carcinogenicity. In summary, organoids are expected to replace traditional in vivo models, facilitate the exploration of toxic effects and action mechanisms of environmental pollutants, and possess promising prospects for accurately evaluating the human health risks posed by pollutants.

However, for organoid models to truly replace traditional exposure models, several limitations and key issues still need to be addressed. Firstly, limited availability of seed cells constitutes the primary bottleneck for organoid culture.

Restricted by large sample demand and ethical regulations, stable acquisition of initial cells for cultivation remains difficult [124]. Cell heterogeneity is a critical pending issue in organoid research. Single-cell sequencing enables precise subtype classification of cells and improves the reliability of molecular mechanism interpretation. In addition, insufficient nutrient supply, shortage of bioactive factors and poor gas exchange hinder the differentiation and maturation of organoids, and it is difficult to precisely control their size, cellular composition and spatial structure. Therefore, it is urgent to establish standardized culture systems for developing pollutant exposure evaluation models with favorable repeatability and accuracy [125]. On the other hand, current organoids lack blood vessels, nerves and immune cells, limiting studies on pollutant metabolism and immunotoxicity and accurate toxicity evaluation [126]. Therefore, 3D bioprinting and organ-on-a-chip technologies can build organoid vascular networks and supplement nerve and immune cells to improve organoid physiological simulation for precise pollutant toxicity testing. Furthermore, organoid construction and culture are affected by cell sources and culture approaches, causing batch-to-batch variation that impairs experimental reproducibility and comparability. Inconsistent manual operations further hamper standardization and restrict organoid applications in toxicity assays [127]. Standardized and automated culture procedures, culture medium and cell selection are essential to lower variation and enhance experimental reproducibility in the future. Finally, organoids possess short lifespan, restricting long-term pollutant exposure tests and relevant researches on pollutant chronic toxicity and chronic disease progression. They fail to satisfy research demands for long-term monitored toxic outcomes like pollutant carcinogenicity [128]. Therefore, novel biomimetic hydrogels are vital to optimize organoid culture microenvironment and prolong organoid lifespan. Such materials support long-term pollutant exposure assays and facilitate precise analysis of pollutant chronic toxicity and chronic disease progression.

6 Conclusions

With their core advantage of simulating the structure and function of human organs, 3D organoids are reshaping the environmental toxicology evaluation system with high biomimetic precision. They effectively address the limitations of traditional 2D cell culture (lack of physiological structure and cell-cell interactions) and animal models (interspecies differences). Although organoids cannot yet fully replace animal models, organoid-based exposure assessment models remain ideal alternative tools for *in vitro* evaluation of the toxic effects of environmental pollutants. These models can cover a variety of organoids, including intestinal, renal, brain, hepatic, and pulmonary organoids. By systematically detecting the toxic responses of different organoids, they provide key support for clarifying the multi-target toxic mechanisms of environmental pollutants. The development of organoid technology not only responds to animal experiment phase-out initiatives but also drives toxicology toward the era of precision through high throughput, low cost, and ethical compliance. Ultimately, it will achieve full-chain breakthroughs from environmental pollutant early warning to personalized

health protection. In general, future research needs to overcome current limitations. By deepening the interdisciplinary integration of 3D organoid technology with environmental toxicology, materials science, molecular biology, bioengineering, and artificial intelligence, we can fully unleash the application potential of organoids in the study of the toxic mechanisms of environmental pollutants. This will provide scientific support for improving the human health risk assessment system and optimizing environmental protection strategies, thereby promoting the high-quality development of undertakings in related fields.

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Declarations

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