

**NEXT-GENERATION TOXICITY TESTING: A COMPARATIVE REVIEW OF
ORGAN-ON-A-CHIP VERSUS TRADITIONAL IN VIVO MODELS**Maitreya Paramkusam¹, Shaik Abdul Azeez^{1,*}¹Department of Pharmacy Practice, Sir C. R. Reddy College of Pharmaceutical Sciences, Eluru – 534007, Andhra Pradesh, India**ABSTRACT**

Objective: To compare organ-on-a-chip (OoC) microphysiological systems with conventional in vivo animal models for preclinical toxicity testing across predictive validity, cost and throughput, moral and regulatory standing, and systemic physiological intricacy.

Methods: This is a narrative, non-systematic comparative review. Sources were identified by directed searches of PubMed, Google Scholar, and the websites of the FDA, EMA and CDSCO, supplemented by hand-searching essential reference lists, focusing on primary validation studies, regulatory announcements, and significant evaluations released 2007–2026; official systematic-review methodology (search strings, date limits, PRISMA option) was not applied.

Results: Standard animal panels predict human toxicity with concordance of approximately 71% overall (63% non-rodents exclusively, 43% rodents solely) [1]. A blinded validation study of a human Liver-Chip reported 87% sensitivity and 100% specificity for drug-induced liver injury (DILI) across 870 chips, three donors and 27 drugs [2]. As of September 2024, the FDA's IStand Pilot Program had accepted a Letter of Intent for this Liver-Chip; qualification remains in progress [3]. OoC platforms otherwise continue to be limited by unfinished standardisation, the absence of immune/endocrine crosstalk, and reduced throughput compared with traditional assays.

Conclusion: OoC platforms already outperform conventional and customary animal models for explicit and well-defined endpoints, most distinctly hepatotoxicity, but continue to be constrained for systemic and persistent toxicity. The domain is converging on a hybrid testing paradigm in which OoC technology increasingly displaces select in vivo studies while animal models retain a narrowing but still vital and indispensable role, at least over the following decade.

Keywords: Organ-On-A-Chip, Microphysiological Systems, New Approach Methodologies, In Vivo Toxicology, Drug-Induced Liver Injury, Preclinical Safety Assessment, Induced Pluripotent Stem Cells, Microfluidics, FDA Modernisation Act

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Abbreviations: OoC, organ-on-a-chip; MPS, microphysiological system; NAM, new approach methodology; PDMS, polydimethylsiloxane; iPSC, induced pluripotent stem cell; TEER, transepithelial/transendothelial electrical resistance; BBB, blood–brain barrier; DILI, drug-induced liver injury; hERG, human Ether-à-go-go-Related Gene; QT, QT interval; NOAEL, no-observed-adverse-effect level; ICH, International Council for Harmonisation; IND, Investigational New Drug; NDA, New Drug Application; ISTAND, Innovative Science and Technology Approaches for New Drugs; NCATS, National Center for Advancing Translational Sciences; DARPA, Defense Advanced Research Projects Agency; OECD, Organisation for Economic Co-operation and Development; PBPK, physiologically based pharmacokinetic; CNS, central nervous system; 3Rs, Replacement, Reduction, Refinement; CDSCO, Central Drugs Standard Control Organisation; CiPA, Comprehensive In Vitro Proarrhythmia Assay; EMA, European Medicines Agency.

1. INTRODUCTION

Every therapeutic agent, industrial chemical, cosmetic ingredient, and agrochemical situated on the market must first clear a gauntlet of toxicity screening intended to safeguard human health. For most of the twentieth and early twenty-first centuries, this gauntlet has been built almost completely around live animal testing: acute and repeat-dose rodent studies, non-rodent persistent toxicology in dogs or non-human primates, reproductive and developmental toxicity panels, and two-year rodent carcinogenicity bioassays. These studies continue embedded in International Council for Harmonisation (ICH) guidelines and are, in most jurisdictions, a legal precondition for first-in-human dosing.

Despite this entrenchment, the translational performance of animal toxicology has been repeatedly questioned. Systematic evaluations of oncology and cardiovascular compounds have found that treatment effects recorded in animal models often fail to reproduce in human trials [4,5], and post-marketing withdrawals continue to occur for hepatotoxicity and cardiotoxicity that animal studies did not flag [6]. Approximately nine in ten drug candidates that enter clinical development fail to reach consent, and failures of preclinical toxicology prediction, alongside effectiveness shortfalls, are consistently cited among the leading reasons for attrition [6,7]. These translational gaps, combined with growing moral objections to animal application and legislative changes such as the 2013 European Union cosmetics testing ban [8] and the 2022 United States

FDA Modernisation Act 2.0 [9], have produced both scientific and regulatory momentum for alternative testing methods jointly termed “new approach methodologies” (NAMs) [10,11].

Among NAMs, organ-on-a-chip (OoC) technology, also called a microphysiological system (MPS) or tissue chip, has attracted particular attention because it aims not merely to supplant an animal model but to rebuild a streamlined, functional unit of human physiology [12,13,14]. An OoC device is commonly a microfluidic chip, frequently fabricated in optically clear polydimethylsiloxane (PDMS), containing one or more hollow microchannels lined with living human cells that are continuously perfused with culture medium under physiologically relevant flow, mechanical strain, or electrical stimulation [12,15]. First demonstrated at organ-level fidelity by Huh and colleagues with a “breathing” lung-on-chip in 2010 [16], the area has since expanded to liver, kidney, heart, gut, skin, blood–brain barrier, and multi-organ “body-on-chip” configurations [17,18,19,20].

This review is a narrative comparison rather than an official methodical review (see Methods above and the Limitations part). It examines OoC platforms against customary *in vivo* toxicity testing across specific, quantifiable domains: predictive concordance with human clinical results, cost and throughput, moral and regulatory acceptability, and physiological integrity; and synthesises organ-specific case evidence, the shifting worldwide regulatory landscape (incorporating an Indian regulatory perspective), and the technical hurdles that continue to restrict OoC adoption. The goal is to give toxicologists, drug development scientists, regulators, and biomedical engineers a present, balanced reference point for deciding where each model class is best deployed.

2. TRADITIONAL IN VIVO TOXICITY TESTING MODELS

2.1 Historical Development and Regulatory Foundations

Systematic animal toxicity testing became systematised in the aftermath of the 1937 sulfanilamide elixir poisoning and, more decisively, the thalidomide tragedy of the late 1950s and early 1960s, which prompted the 1962 Kefauver-Harris Amendment in the United States and similar and comparable legislation elsewhere requiring proof of safety, comprising reproductive and teratogenic safety, before marketing [10]. Over later decades, the International Council for Harmonisation codified a uniform and consistent battery of studies: ICH M3(R2) for nonclinical safety studies, S1 for carcinogenicity, S5 for reproductive toxicity, and S7A/S7B for safety pharmacology; this remains the backbone of worldwide regulatory submissions today. More recently, the ICH S1B(R1) addendum introduced a weight-of-evidence approach that allows the customary two-year rat carcinogenicity study to be waived in specified situations where an integrated assessment of other data is judged sufficient, marking one of the first instances in which a core ICH guideline officially reduced a long-standing animal testing condition [21].

2.2 Standard In Vivo Study Designs

A standard preclinical toxicology package for a small-molecule drug commonly proceeds through multiple tiers of rising length and species intricacy:

- Acute toxicity studies establishing single-dose lethality and maximum tolerated dose, normally in rodents.
- Repeat-dose (sub-acute and sub-chronic) studies of two to thirteen weeks in a rodent and a non-rodent species, generating no-observed-adverse-effect-level (NOAEL) data.
- Chronic toxicity studies of six months to one year, primarily for drugs intended for long-term human application.
- Genotoxicity, reproductive and developmental toxicity, and juvenile toxicity studies.
- Two-year rodent carcinogenicity bioassays for drugs with anticipated persistent human exposure, now subject to the ICH S1B(R1) weight-of-evidence waiver in specified instances [21].
- Safety pharmacology studies assessing cardiovascular (hERG and in vivo QT), respiratory, and central nervous system effects.

2.3 Strengths of Animal Models

Animal models retain genuine, non-trivial benefits that clarify their persistent regulatory significance [7]. They provide an intact, systemic physiology in which the endocrine, immune, nervous, and circulatory systems interact just as they would in a whole organism, permitting detection of toxicities that arise solely from inter-organ signalling; for case, hepatorenal syndromes, systemic immune-mediated hypersensitivity, or drug effects mediated through the hypothalamic-pituitary axis [7,17]. Whole-animal studies moreover capture persistent, cumulative, and delayed toxicities over months to years, incorporate normal ageing and comorbidity-like variability, and benefit from an immense historical control database spanning decades of regulatory experience, which underpins statistical confidence in interpreting new outcomes [10].

2.4 Limitations of Animal Models

The central deficiency of animal toxicology is interspecies discordance [6,7]. Rodents and humans differ significantly in drug-metabolising enzyme expression (notably cytochrome P450 isoforms), plasma protein binding, immune receptor repertoires, and organ-specific drug transporters, so a compound metabolised safely in rats may generate a reactive, hepatotoxic metabolite in humans, or vice versa [6]. This is quantified directly by the ILSI-HESI multinational concordance survey of Olson and colleagues, which compared 150 pharmaceutical compounds and 221 human toxicity events against paired animal study data: the true-

positive concordance rate between animal toxicology results and human toxicity was approximately 71% when rodent and non-rodent species were combined, declining to 63% for non-rodent species alone and 43% for rodent species solely, with the weakest concordance for cutaneous toxicity and the strongest for hematological, gastrointestinal, and cardiovascular toxicity [1]. Retrospective analyses of withdrawn drugs have individually found that a substantial fraction of human hepatotoxicity and cardiotoxicity events was not predicted by standard animal panels [4,5,6]. Compounding this, animal studies are slow; a complete and entire chronic and carcinogenicity package can take three to four years; and expensive: Fabre and colleagues, writing in 2014, place the direct cost of a single two-year rat carcinogenicity study in the range of approximately one to four million U.S. dollars at 2014 pricing, a figure that has probably risen with succeeding inflation in contract inquiry and animal husbandry costs [22]. Ethical objections under the 3Rs framework (Replacement, Reduction, Refinement), together with legislative constraints such as the EU's 2013 cosmetics animal-testing marketing ban [8] and the momentum behind the FDA Modernisation Acts [9], have further eroded the default assumption that animal data are either essential and required or sufficient.

3. ORGAN-ON-A-CHIP TECHNOLOGY

3.1 Engineering Principles and Microfluidics

Organ-on-a-chip devices are designed to reproduce, at microscale, the tissue–tissue interfaces, mechanical microenvironment, and biochemical gradients that living cells experience *in vivo* but that are absent from standard flat, static Petri-dish culture [12,13]. Most designs employ two or more parallel microchannels, frequently separated by a thin, porous, extracellular-matrix-coated membrane, permitting one channel to house organ-specific parenchymal cells (for example, alveolar epithelium, hepatocytes, or renal tubular cells) while a neighbouring channel houses a vascular endothelial layer perfused with a blood-surrogate medium [12,16]. Continuous microfluidic perfusion delivers nutrients and test compounds while removing metabolic waste, mimicking physiological shear stress and clearance kinetics that static culture can not reproduce [15]. Many devices additionally impose cyclic mechanical strain (to mimic breathing, peristalsis, or cardiac contraction), electrical pacing, or vacuum-actuated stretching to recreate the mechanical forces experienced by the corresponding human organ [16,23]. Figure 1 illustrates a generalised dual-channel architecture representative of lung-, gut-, and kidney-on-chip devices.

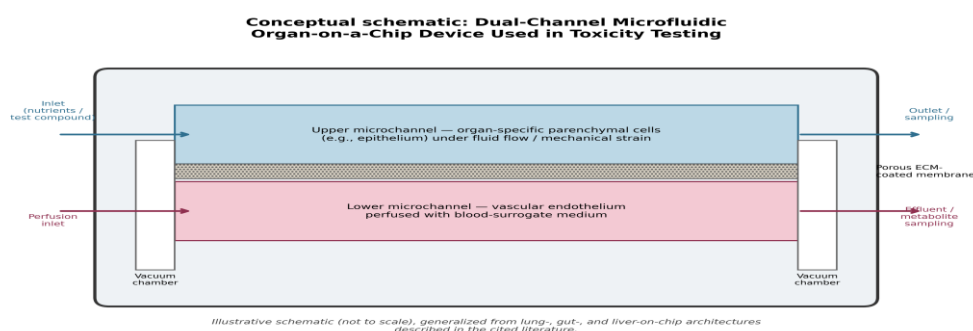


Fig. 1: Conceptual schematic of a dual-channel microfluidic organ-on-a-chip device, showing the upper parenchymal channel, porous membrane, and lower vascular channel with independent perfusion and sampling ports. [12,16,24]

3.2 Types of Organ-on-a-Chip Platforms

A wide variety of single-organ chips has now been reported, each targeting the toxicological endpoints most relevant to a given organ system:

- Liver-on-chip; hepatocyte and non-parenchymal (Kupffer, stellate, sinusoidal endothelial) co-cultures for drug-induced liver injury and metabolic clearance [2,25,26].
- Lung-on-chip; alveolar epithelium and pulmonary microvascular endothelium under cyclic mechanical strain, used for inhalation toxicity and pulmonary oedema modelling [16].
- Kidney-on-chip; proximal tubule epithelium under fluid shear stress, used to model nephrotoxicant-induced tubular injury and transporter-mediated drug handling [27].
- Heart-on-chip; induced pluripotent stem cell (iPSC)-derived cardiomyocytes on flexible substrates for contractility, arrhythmia, and cardiotoxicity assessment [23].
- Gut-on-chip; intestinal epithelium, frequently with peristalsis-like motion and commensal microbiota, for absorption and gut-barrier toxicity [27,28].
- Blood–brain barrier (BBB)-on-chip; brain microvascular endothelium, pericytes, and astrocytes for neurotoxicity and CNS drug-penetration studies [27].
- Multi-organ or “body-on-chip” systems; two or more organ modules fluidically interconnected to capture inter-organ metabolism and systemic pharmacokinetics [18,28,29,30].

3.3 Fabrication, Materials, and Cell Sourcing

Most first-generation OoC devices are soft-lithography-moulded from polydimethylsiloxane (PDMS) because of its optical transparency, gas permeability, and biocompatibility, though PDMS is known to absorb small hydrophobic drug molecules,

which can confound dose–response measurements and has driven interest in alternative thermoplastics such as polystyrene or cyclic olefin copolymer for later-generation, industrially scalable devices [15]. Cell sourcing has shifted increasingly toward human prompted pluripotent stem cell (iPSC)-derived tissue, which enables patient-specific or population-diverse genetic backgrounds to be merged, a capability with no equivalent in animal models [31].

3.4 Readouts, Sensors, and Data Integration

Because OoC devices are optically accessible, they support real-time, non-destructive readouts comprising transepithelial/transendothelial electrical opposition (TEER) for barrier integrity, integrated oxygen and pH microsensors, fluorescence and impedance-based viability assays, and effluent sampling for metabolomic or cytokine profiling [32]. Several groups have embedded these sensors directly into the chip substrate to permit continuous, automated observation over multi-week culture periods, moving the technology closer to the throughput and standardization expected in regulatory toxicology [32].

4. COMPARATIVE ANALYSIS

4.1 Predictive Accuracy and Translational Validity

The most consequential comparison between the two paradigms concerns how dependably each forecasts a toxic outcome in humans, and this part reports the real and genuine figures rather than a qualitative description. The ILSI-HESI concordance survey of Olson and colleagues, covering 150 compounds and 221 human toxicity events, found a true-positive concordance rate between animal results and human toxicity of 71% when rodent and non-rodent data were combined, 63% for non-rodent species solely, and 43% for rodent species solely [1]. By contrast, a big and substantial blinded validation study of the human Liver-Chip; 870 individual chips, built from three hepatocyte donors and

challenged with a blinded panel of 27 drugs with known hepatotoxic or non-toxic behaviour; reported 87% sensitivity and 100% specificity for drug-induced liver injury, compared favourably by the identical study to the historical performance of animal models and of 3D human hepatocyte spheroid culture on the identical compound panel [2]. Figure 2 and Table 1 present these values directly. The two studies used distinct compound panels, dissimilar organ

endpoints, and distinct performance metrics (concordance versus sensitivity/specificity), so the figures are not precisely equivalent and should not be read as a single head-to-head trial; this caveat is revisited in the Limitations section.

Comparable, if less extensive, validation work exists for cardiac, renal, pulmonary, and blood–brain-barrier chips [23,27], summarized qualitatively in Table 3.

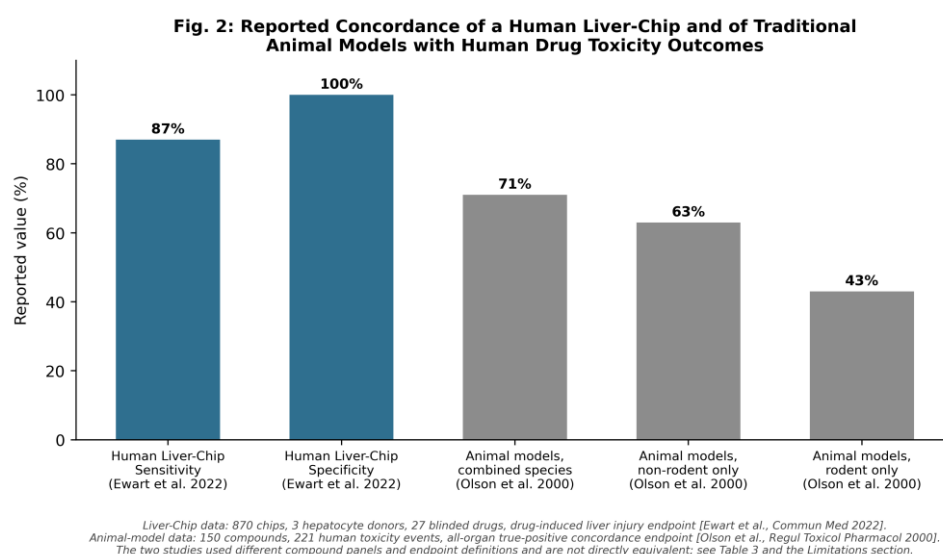


Fig. 2: Reported concordance of a human Liver-Chip and of traditional animal models with human drug toxicity outcomes [1,2].

Model / Study	Endpoint	Reported Value(s)	Study Parameters
Human Liver-Chip (Ewart et al., 2022)	Drug-induced liver injury (DILI)	Sensitivity 87%; specificity 100%	870 Liver-Chips; 3 hepatocyte donors; 27 blinded drugs
Traditional animal models, combined rodent + non-rodent (Olson et al., 2000)	All-organ human toxicity, true-positive concordance	71% combined; 63% non-rodent alone; 43% rodent alone	150 compounds; 221 human toxicity events; 12-company, ILSI-HESI survey

Table 1: Source data underlying Figure 2 concordance and validation-study parameters for the human Liver-Chip and for traditional animal toxicology [1,2].

4.2 Cost and Time Efficiency

Traditional in vivo toxicology packages are resource-intensive: animal husbandry, veterinary oversight, histopathology, and long study periods accumulate substantial direct costs, and the sequential nature of tiered animal studies extends preclinical timelines by years [22]. OoC platforms shorten various of these steps because devices can be run in parallel, generate

readouts within days to a few weeks, and avoid animal housing and breeding overhead; various sector cost-modelling analyses document decreases in per-compound preclinical safety spending when OoC assays are used to triage candidates before committing to total animal studies [2,15]. However, OoC devices carry their own costs; specialized microfabrication, iPSC-derived cell lines, perfusion instrumentation, and highly trained personnel, which now make

small-scale adoption capital-intensive even though per-run costs fall as throughput scales [15,22]. Figure 3 illustrates representative,

order-of-magnitude timeline differences across four preclinical stages.

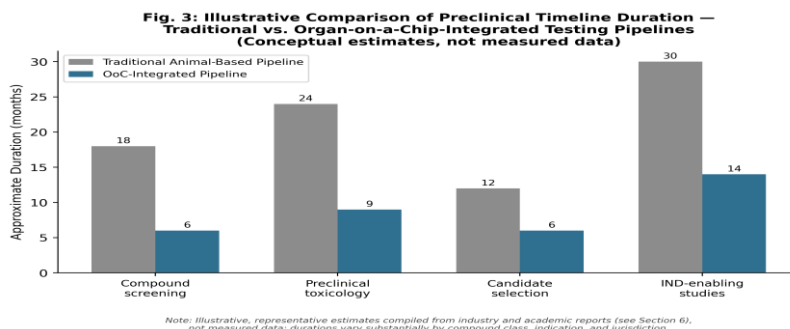


Fig. 3: Comparison of approximate stage duration in a traditional animal-based preclinical pipeline versus an OoC-integrated pipeline. [15,22]

4.3 Ethical and Regulatory Considerations

Animal toxicology testing is subject to rising moral scrutiny under the 3Rs framework and to jurisdiction-specific legislative constraints, most notably the European Union's 2013 ban on marketing cosmetics tested on animals [8] and growing public and organizational pressure to reduce animal application in biomedical studies widely. OoC platforms sidestep these moral objections completely, since no sentient animal is used, and align with the stated direction of regulatory policy: the U.S. FDA Modernization Act 2.0 (2022) removed the statutory condition that preclinical safety data be generated in animals, allowing cell-based assays, organ chips, and computational models as admissible nonclinical evidence [9], and the follow-on Modernization Act 3.0, introduced in Congress in February 2024, seeks to direct the FDA to build an official qualification pathway for such strategies; as of this writing, Act 3.0 has not been enacted [33].

The regulatory position on organ-chip qualification specifically necessitates precise description. On 24 September 2024, the FDA's Centre for Drug Evaluation and Research accepted the first Letter of Intent into the Innovative Science and Technology Approaches for New Drugs (ISTAND) Pilot Program for an organ-on-a-chip technology: a human Liver-Chip intended to evaluate the

relative risk of drug-induced liver injury for applicant drugs in support of Investigational New Drug (IND) submissions [3]. Acceptance of a Letter of Intent is the first of three stages in the ISTAND process; the developer must then finish a Qualification Plan demonstrating reproducibility across laboratories and hepatocyte donors before an instrument can be assessed for Full Qualification [3,34]. As of the date of this review, the Liver-Chip assay remains in this qualification process and has not accomplished it; no organ-on-a-chip technology has yet accomplished Full Qualification under ISTAND. The ISTAND pilot program itself has been established as a lasting and enduring FDA drug-development-tool qualification pathway, rather than remaining a time-limited pilot [34]. Nonetheless, regulatory maturity for OoC remains far behind that of animal testing: reference historical control databases, harmonised validation protocols, and inter-laboratory reproducibility standards are still under active development through initiatives such as the U.S. National Institutes of Health/NCATS Tissue Chip for Drug Screening program and OECD NAMs direction efforts [11,35].

4.4 Throughput and Scalability

Conventional in vitro assays (2D cell culture, high-throughput screening plates) significantly outperform both animal studies

and OoC devices in raw throughput, screening tens of thousands of compounds per week. Animal studies sit at the reverse extreme, normally assessing single-digit-to-low-tens of compounds per study given cost, husbandry, and moral review limitations [4]. OoC platforms occupy an intermediate position: individual chips are labour- and instrumentation-intensive to run relative to easy and straightforward 2D assays, but automation, robotic fluidic coupling of various chips, and multiplexed chip arrays are gradually closing this gap, permitting medium-throughput secondary screening that was earlier solely practical and workable in animals [18,19,29].

4.5 Systemic Complexity versus Modular Precision

Figure 4 presents a qualitative, multi-criteria comparison synthesising the trade-offs

discussed above [17,31]; the scoring is a conceptual, author-assigned example rather than measured data. Animal models score extremely on systemic intricacy, inter-organ crosstalk, and regulatory maturity, indicating their intact physiology and decades of collected regulatory approval, but score poorly on human relevance, moral acceptability, and cost-efficiency. OoC platforms invert this profile: they offer high human physiological relevance and moral standing but, in most present implementations, model solely one or a small number of interconnected organ modules and thus can not yet capture whole-body phenomena such as systemic immune responses, endocrine feedback loops, or ageing-related comorbidity [11,17].

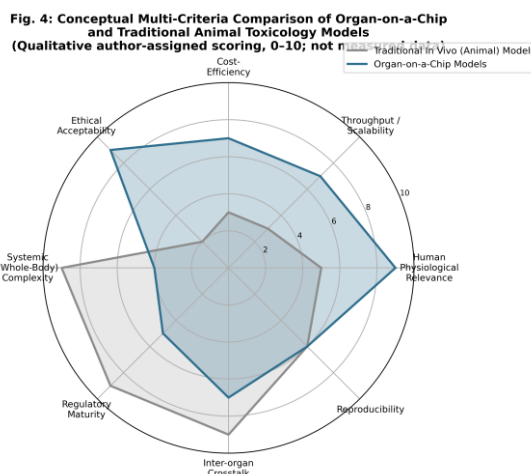


Fig. 4: Conceptual multi-criteria comparison of organ-on-a-chip and traditional animal toxicology models (qualitative, author-assigned scoring, 0–10; not measured data) [17,31].

4.6 Summary Comparison Table

Criterion	Traditional In Vivo (Animal) Models	Organ-on-a-Chip (OoC) Models
Human physiological relevance	Limited by interspecies differences in metabolism, immunity, and receptor biology	High — built from human (often patient-derived iPSC) cells
Systemic / whole-body complexity	High — intact endocrine, immune, nervous, circulatory systems	Low to moderate — improving with multi-organ chip coupling
Typical study duration	Weeks to years (chronic and carcinogenicity studies)	Days to several weeks per assay

Criterion	Traditional In Vivo (Animal) Models	Organ-on-a-Chip (OoC) Models
Relative cost per study	High (husbandry, veterinary oversight, long duration); ~US\$1–4 million for a 2-year rat carcinogenicity study, 2014 pricing (Fabre et al.)	Moderate; falls with scale, but device/consumable and iPSC costs are non-trivial
Throughput	Very low (ethical/cost limits on animal numbers)	Low to moderate, improving with automation
Ethical burden	Significant; subject to 3Rs oversight and legislative limits	Minimal; no sentient animal used
Regulatory status (Aug 2026)	Long-established default requirement in ICH guidelines	Letter of Intent accepted into FDA IStand (liver, Sept 2024); qualification in progress, not yet complete
Chronic / lifetime toxicity capture	Well established (e.g., 2-year rodent bioassay; waivable in defined cases under ICH S1B(R1))	Limited; long-term chip culture stability remains a technical challenge.
Inter-individual genetic diversity	Limited to inbred/outbred colony strains	High potential via diverse patient-derived iPSC lines
Reproducibility across sites	Well characterized historically, but interspecies variability persists	Improving but not yet fully standardized across laboratories

Table 2: Summary comparison of traditional in vivo animal models and organ-on-a-chip models across key toxicological, operational, and regulatory criteria [3,6,17,22].

5. ORGAN-SPECIFIC CASE STUDIES

5.1 Hepatotoxicity: Drug-Induced Liver Injury (DILI)

DILI are the most thoroughly confirmed OoC application and a leading reason for clinical trial failure and post-marketing drug withdrawal; DILI is commonly cited as contributing to about one-fifth of post-marketing drug withdrawals and approximately one-eighth of clinical trial failures, stressing the value of enhancing its preclinical detection [2,6]. Human liver-chip platforms including hepatocytes, Kupffer cells, stellate cells, and liver sinusoidal endothelial cells have been tested in blinded panels containing dozens of compounds with known human hepatotoxic results, reproducibly differentiating hepatotoxic from non-hepatotoxic agents; incorporating various compounds that had passed standard animal toxicology and exclusively revealed liver injury in human trials or post-marketing monitoring [2,25,26]. As described in Section 4.3, this body of evidence underpinned the FDA's September 2024 approval of a Letter of Intent for a liver-chip DILI assay into the IStand Pilot Program;

a crucial and vital step toward regulatory qualification, though qualification itself remains in progress, rather than full and comprehensive [3,24]. This denotes the clearest existing proof of concept that OoC data can meet or surpass animal-model performance for a distinct, well-defined endpoint, while exemplifying how long the total and whole regulatory qualification pathway takes even for the most mature OoC application.

5.2 Cardiotoxicity

Heart-on-chip platforms employing iPSC-derived cardiomyocytes on flexible, electrode-instrumented substrates can quantify contractile force, beat rate, and electrophysiological parameters similar to and comparable to a human electrocardiogram, offering a route to detect QT-prolongation and pro-arrhythmic risk with truly human ion-channel biology [23]. This complements the Comprehensive In Vitro Proarrhythmia Assay (CiPA) initiative, the primary regulatory-science programme reshaping cardiac safety assessment away from reliance on the hERG-channel assay plus in vivo telemetry solely, toward an

integrated paradigm merging in silico modelling, human stem-cell-derived cardiomyocyte assays, and directed ion-channel profiling; the related ICH S7B/E14 Questions and Answers document formalises how this paradigm is applied in regulatory submissions [36]. Because cardiotoxicity frequently manifests solely after persistent dosing, present chip culture-longevity limitations mean heart-on-chip platforms are most established for acute and sub-acute electrophysiological screening within the broader CiPA framework, rather than substituting long-duration in vivo cardiac safety studies outright [20,23].

5.3 Nephrotoxicity

Kidney-on-chip devices modelling the proximal tubule under physiological shear stress have demonstrated enhanced sensitivity to nephrotoxics such as cisplatin and specific aminoglycoside antibiotics relative to static human tubular cell culture, by better maintaining transporter expression and polarised epithelial function that is swiftly lost in traditional culture [27]. Because kidney injury often depends on active transporter-mediated drug collection, duplicating physiological flow has proven especially significant for this organ [27,28].

5.4 Pulmonary Toxicity

The initial “breathing” lung-on-chip demonstrated that cyclic mechanical strain mimicking respiration meaningfully alters epithelial and endothelial responses to inhaled particulates and germs compared to static culture [16], and successor devices have been used to model pulmonary oedema, inflammatory cytokine responses, and nanoparticle toxicity with outputs that are challenging to obtain non-invasively in live animals [13,16].

5.5 Neurotoxicity and the Blood–Brain Barrier

Blood–brain-barrier chips integrating brain microvascular endothelium, pericytes, and astrocytes allow direct, human-relevant measurement of compound permeability and neuroinflammatory response, tackling a long-standing translational gap since rodent BBB transporter expression and tight-junction biology differ meaningfully from human [27]. These platforms continue at a previous stage of validation than liver- or lung-chips but are examined as high-priority given the historically poor translational record of CNS drug development [27,28].

Organ System	Representative Chip Design	Primary Toxicological Endpoint	Validation Maturity (2026)
Liver	Hepatocyte + Kupffer + stellate + sinusoidal endothelial co-culture	Drug-induced liver injury (DILI), metabolic clearance	Letter of Intent accepted into the FDA ISTAND programme; qualification in progress
Heart	iPSC-cardiomyocytes on flexible electrode substrate	Contractility, arrhythmia, QT prolongation (CiPA-aligned)	Moderate — acute/sub-acute screening
Kidney	Proximal tubule epithelium under shear flow	Tubular injury, transporter-mediated toxicity	Moderate
Lung	Alveolar epithelium + microvascular endothelium, cyclic strain	Inhalation toxicity, pulmonary oedema, inflammation	Moderate to high
Brain / BBB	Brain endothelium + pericytes + astrocytes	Neurotoxicity, CNS drug penetration	Early to moderate
Gut	Intestinal epithelium with peristaltic motion ± microbiota	Absorption, gut-barrier toxicity	Moderate
Multi-organ (“body-on-chip”)	2–4 interconnected organ modules	Systemic pharmacokinetics, inter-organ metabolism	Early

Table 3: Organ-specific organ-on-a-chip platforms, their primary toxicological application, and approximate validation maturity as of 2026 [2,3,16,23,27,28].

6. REGULATORY LANDSCAPE AND ADOPTION

6.1 United States

Regulatory approval is the single strongest determinant of whether OoC technology will meaningfully displace animal testing, and momentum has accelerated markedly since 2022. The joint NIH–FDA–DARPA Microphysiological Systems Program, launched in 2011, founded the first scientific and regulatory-science foundation for OoC validation [22,35]. The landmark FDA Modernisation Act 2.0, signed into law in December 2022, amended the Federal Food, Drug, and Cosmetic Act to supplant the phrase “preclinical tests (comprising tests on animals)” with “nonclinical tests,” explicitly specified to contain cell-based assays, organ chips, microphysiological systems, bioprinters, and computer modelling [9]. Critically, this legislation allows but does not mandate the application of non-animal data; sponsors retain discretion over which evidence package best supports a given regulatory submission [9]. FDA Modernisation Act 3.0, introduced in February 2024, would lead the agency to build an official qualification, review, and routine-acceptance pathway for NAMs, but had not been enacted as of the date of this review [33].

As described in Section 4.3, on 24 September 2024, the FDA’s ISTAND Pilot Program accepted its first organ-on-a-chip Letter of Intent for a human Liver-Chip DILI assay, which is proceeding through the Qualification Plan stage rather than possessing attained Full Qualification [3]. In April 2025, the FDA published its Roadmap to Reducing Animal Testing in Preclinical Safety Studies, outlining a stepwise plan to reduce reliance on animal testing for monoclonal antibodies and other biologics in favour of confirmed NAMs, incorporating organ-on-a-chip systems, computational modelling, and advanced in vitro assays [37].

6.2 European Union and United Kingdom

Outside the United States, the European Union has taken parallel steps. Its long-standing prohibition on marketing animal-tested cosmetics, in force since 2013, has already compelled extensive and prevalent adoption of non-animal options in that sector [8]. More widely, the European Parliament’s resolution of 16 September 2021 called on the European Commission to develop an EU-wide action plan to accelerate the transition to inquiry, regulatory testing, and education that does not depend on animals, comprising a roadmap of coordinated actions with specified 3Rs benchmarks across the Union [38]. The European Medicines Agency has correspondingly expanded its engagement with NAM developers through briefing gatherings and its Qualification of New Methodologies procedure, and proceeds to encourage 3Rs-compliant strategies through its Innovation Task Force, while noting that data from NAMs can now be excluded from official regulatory decision-making unless individually qualified for a specified setting of application [39]. The Organisation for Economic Co-operation and Development has, in parallel, been evolving direction documents for qualifying microphysiological systems within its broader NAMs framework, intended to support mutual approval of data across OECD member jurisdictions [11].

6.3 Indian Regulatory Perspective (CDSCO)

India has shifted in step with this worldwide shift. The Central Drugs Standard Control Organisation’s New Drugs and Clinical Trials Rules, 2019 set out, in their First and Second Schedules, the nonclinical data requirements underpinning new drug and clinical trial applications in India [40]. On 9 March 2023, the Ministry of Health and Family Welfare notified an amendment to these rules (G.S.R. 175(E)) that explicitly allows the application of non-animal and human-relevant procedures; incorporating

3D organoids, 3D bioprinted tissue, organ-on-a-chip systems, and advanced computational approaches; to generate the nonclinical safety and effectiveness data required for new drug applications, officially opening a regulatory route for OoC-derived evidence in Indian drug development for the first time [41]. This sits alongside India's existing Committee for Control and Supervision of Experiments on Animals (CPCSEA) framework, which governs the breeding of and testing on laboratory animals under the Breeding of and Experiments on Animals (Control and Supervision) Rules [42], and India's 2014 prohibition, under the Drugs and Cosmetics Rules, on testing cosmetics on animals and on importing animal-tested cosmetics; among the earliest such bans globally, predating the EU's equivalent prohibition by more than a year in some respects [43]. Microphysiological systems study and system in India continue at an initial stage relative to the United States and European Union, concentrated in a small number of premier academic institutes and biotechnology incubators, with limited commercial CRO capability as of 2026; the 2023 CDSCO amendment however provides Indian academic and sector groups a specified regulatory pathway to build toward, and denotes a genuine chance for Indian pharmaceutical sciences programmes to contribute to this area.

Taken together, the regulatory trajectory across important and significant jurisdictions favours an expanding, though still supportive rather than replacement, role for OoC evidence over the approaching decade [11,17].

7. CHALLENGES AND LIMITATIONS OF ORGAN-ON-A-CHIP TECHNOLOGY

Despite genuine progress, various technical and systemic obstacles continue to restrict OoC adoption at scale [11,17].

- **Standardisation:** chip geometry, materials, cell sourcing, and readout protocols differ broadly between academic and commercial developers,

complicating cross-laboratory reproducibility and regulatory qualification [11,15].

- **Material interruption:** PDMS, the primary and principal chip material, absorbs small hydrophobic drug molecules, which can artificially reduce effective test-compound concentrations and confound dose–response interpretation [15].
- **Absent systemic physiology:** most single-organ chips lack immune cell populations, endocrine feedback, bone marrow, and adipose tissue, precluding detection of toxicities that rely on whole-body signalling [11,17].
- **Vascularisation and long-term culture stability:** sustaining functional, perfusable microvasculature and feasible tissue architecture over the weeks to months required for persistent toxicity assessment remains technically demanding [29,30].
- **Validation strain and cost of adoption:** developing the historical reference databases and inter-laboratory validation studies required for total and whole regulatory qualification is itself a multi-year, resource-intensive project, illustrated by the Liver-Chip's multi-year progression through the IStand pathway, and specialised fabrication and iPSC culture skills raise the obstacle to entry for smaller organisations [3,11,35].
- **Throughput ceiling:** even with automation, OoC assays continue to be lower-throughput than traditional high-throughput screening, restricting their contemporary and ongoing role to secondary or confirmatory testing rather than primary large-library screening [18,19].
- **Regulatory conservatism and inertia:** because animal data underpins decades of precedent and organisational familiarity, both sponsors and reviewers may default to established animal packages even where an OoC assay would be scientifically fitting and right [9,37].

8. FUTURE DIRECTIONS

8.1 Multi-Organ and Whole-Body-on-Chip Systems

Fluidically interconnected multi-organ platforms- coupling, for example, gut, liver, kidney, and skin modules, or vascularized multi-chip arrays with robotic perfusion- have already demonstrated the capability to reproduce human-like pharmacokinetic parameters and inter-organ metabolite transfer [28,29,30], and represent the clearest technical path toward closing the systemic-complexity gap identified in Section 4.5.

8.2 Integration with Artificial Intelligence and Computational Modelling

Machine learning models trained on chip-derived imaging, sensor, and omics data are increasingly used to predict toxicity signatures, while physiologically established pharmacokinetic (PBPK) and quantitative systems toxicology models are being linked to chip outputs to extrapolate organ-level chip data to whole-body human dose-response predictions, partly compensating for the lack of total and whole systemic physiology on a single device [32,44].

8.3 Patient-Derived iPSCs and Personalised Toxicology

Because OoC devices can be seeded with iPSCs derived from genetically varied and distinct donor populations, incorporating “cell village” pooling plans that culture several donor lines together, the technology initiates a route to population-representative and even individualised toxicity prediction that is structurally inaccessible to inbred animal colonies, with direct relevance to pharmacogenomic safety signals and precision medicine [31]. This is conceptually related to, but technically distinct from, patient-derived organoid models: three-dimensional, self-organizing cell aggregates such as those used to model treatment response in metastatic gastrointestinal cancer [45] capture some patient-specific genetic and phenotypic variety but generally lack the designed vasculature, fluid flow, and mechanical microenvironment that define a

microphysiological system in the sense used throughout this review; the two techniques are increasingly combined, for illustration by seeding organoids within a chip’s parenchymal channel, but should not be treated as interchangeable.

8.4 Standardisation and Consortium-Led Validation

Continued investment through programs such as the NIH/NCATS Tissue Chip for Drug Screening initiative, sector consortia, and official OECD advice development will be vital and indispensable to establish the harmonised protocols, reference compound panels, and inter-laboratory reproducibility data required for OoC assays to progress from supportive to potentially primary regulatory evidence for a broader range of endpoints [11,35].

8.5 A Hybrid Testing Paradigm

The weight of present and existing evidence implies that OoC technology is improbable and doubtful to fully supplant animal toxicology within the approaching decade, but is instead converging with computational modeling and standard in vitro assays into a tiered, hybrid testing paradigm: high-throughput 2D/3D in vitro and computational screening for initial triage, organ-specific OoC assays for mechanistic and organ-targeted endpoints where human relevance is essential (as already realized, in part, for DILI), and a narrowing set of animal studies reserved for systemic, persistent, and reproductive endpoints that present chip technology can not yet adequately capture [17,20].

9. CONCLUSIONS

Organ-on-a-chip technology has advanced from a proof-of-concept lung-on-chip in 2010 [16] to an organ-on-a-chip assay accepted into the FDA’s ISTAND qualification pathway for drug-induced liver injury in 2024 [3], representing one of the swiftest translational paths in modern toxicology, even though that assay’s qualification remains in progress rather than fully and comprehensive. The comparative

evidence assembled in this review signifies that OoC platforms already match or exceed conventional animal models for precise and particular, well-characterized endpoints; most distinctly hepatotoxicity, where a confirmed Liver-Chip reported 87% sensitivity and 100% specificity against a 71% combined-species concordance benchmark for animal models [1,2]; while remaining restricted by limited systemic physiological intricacy, partial and unfinished standardization, and a still-developing regulatory evidence foundation for other endpoints [11,17]. Traditional *in vivo* models, in turn, retain irreplaceable strengths for capturing persistent, systemic, and multi-organ toxicity but carry well-documented translational, moral, cost, and throughput limitations [4,5,6,7]. Rather than a binary replacement narrative, the most defensible reading of the present evidence is that toxicity testing is entering a hybrid era in which OoC platforms gradually absorb a growing portion of organ-specific and mechanistic testing, supported by an increasingly permissive regulatory framework across the United States, European Union, and India [9,38,41], while animal studies persist; in reduced number and more targeted application; for the systemic and persistent endpoints that microphysiological systems can not yet fully rebuild. Continued investment in standardisation, multi-organ integration, and computational bridging will determine how quickly this balance proceeds to move [17,20,35].

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AUTHOR CONTRIBUTIONS

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ETHICS STATEMENT

Not applicable — this review did not involve human participants or animals.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this review. Neither author has any financial or consulting relationship with Emulate, Inc., CN Bio, or any other commercial developer of organ-on-a-chip technology named in this review.

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