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Perspective

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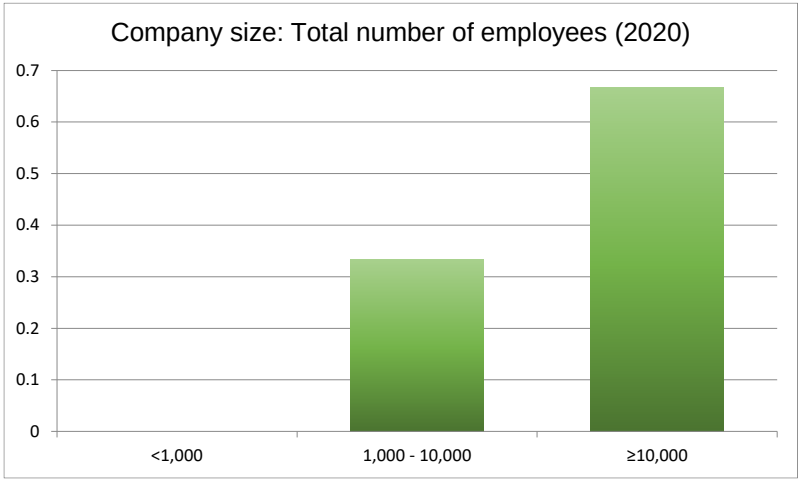
# The evolving role of investigative toxicology in the pharmaceutical industry

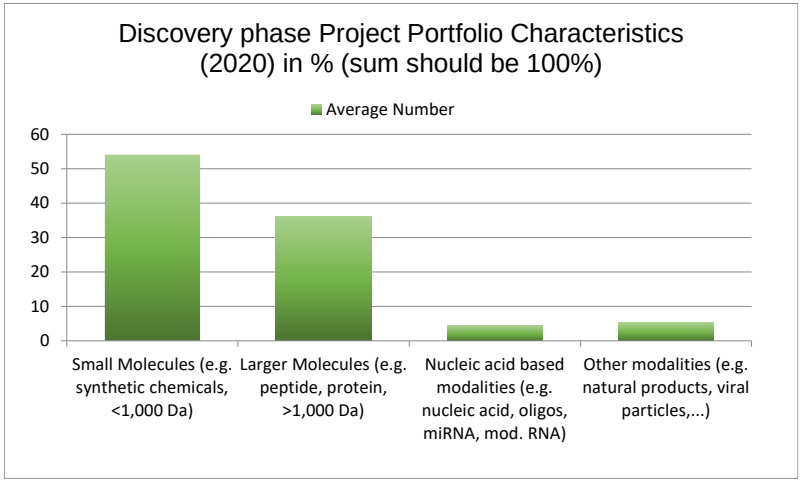
In the format provided by the authors and unedited

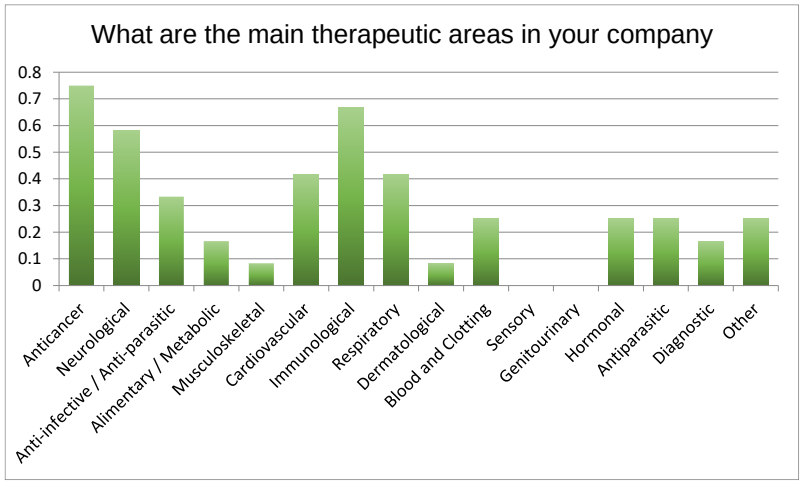
**Investigative Toxicology Leader Forum 2020 survey**

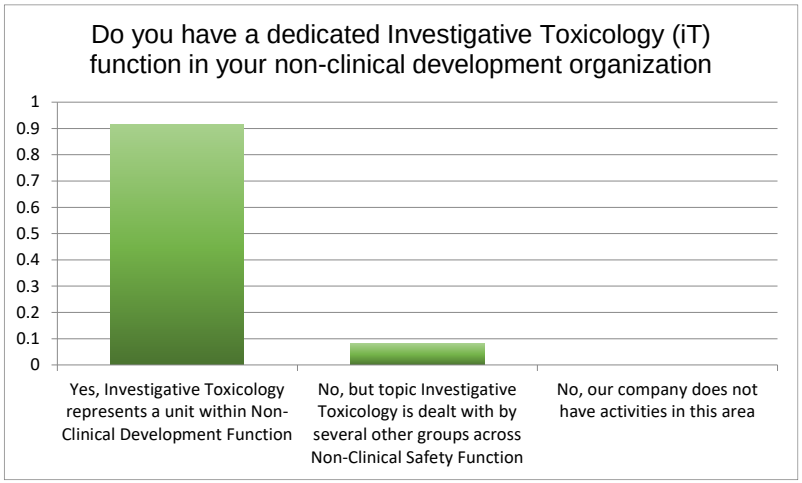
This 2020 survey, following the one of 2015, was conducted by the authors of this publication within their respective 14 mid-to-large size pharmaceutical companies. Further details are provided in Box 1 of the article.

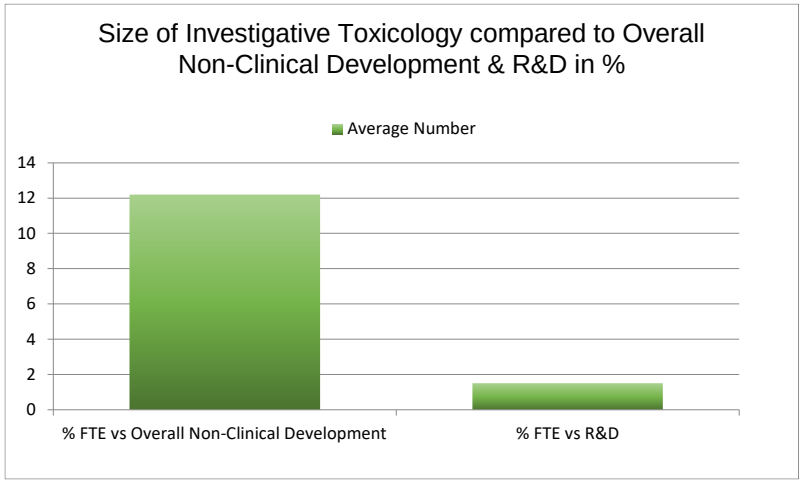
Depending on the questions, replies were either categorical (e.g., yes or no) or actual number (e.g., percentages) or subjective appreciations about the topic being surveyed, in which case, the data were usually averaged where possible. The questions are stated in the title of each graph.

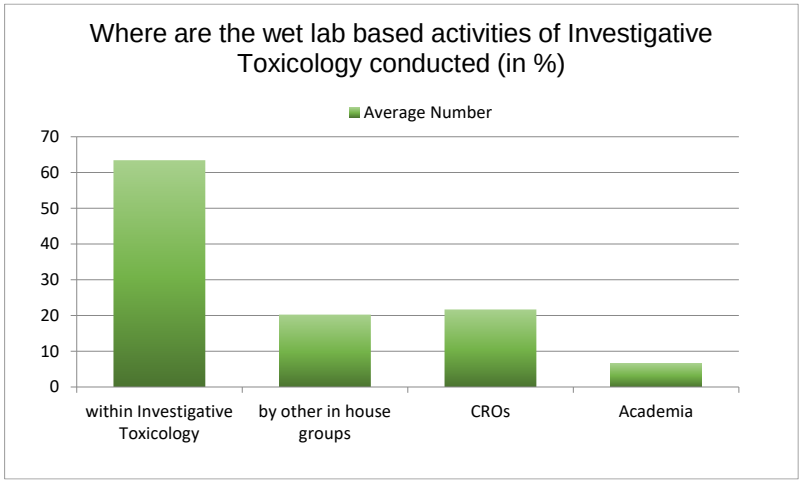


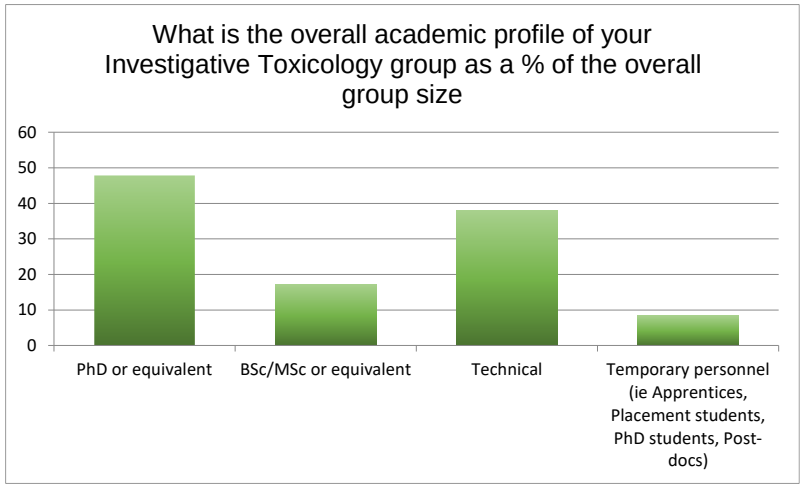



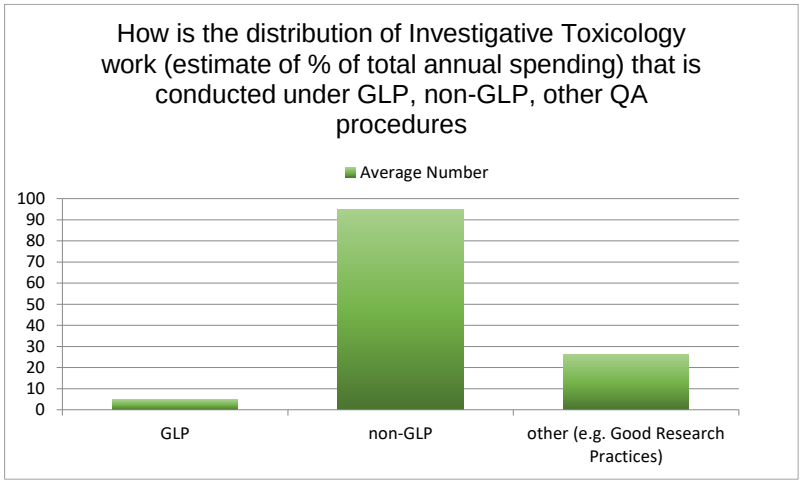



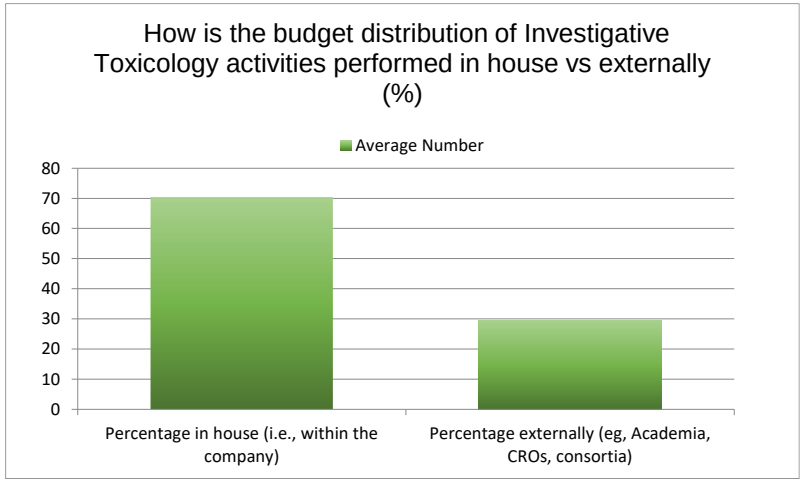



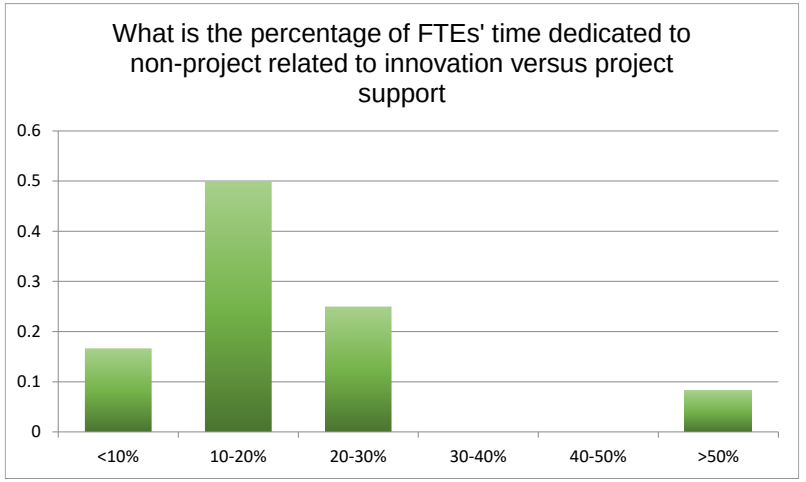

Size of Investigative Toxicology compared to Overall Non-Clinical Development & R&D in %
Average Number
14
12
10
8
6
4
2
0
% FTE vs Overall Non-Clinical Development
% FTE vs R&D

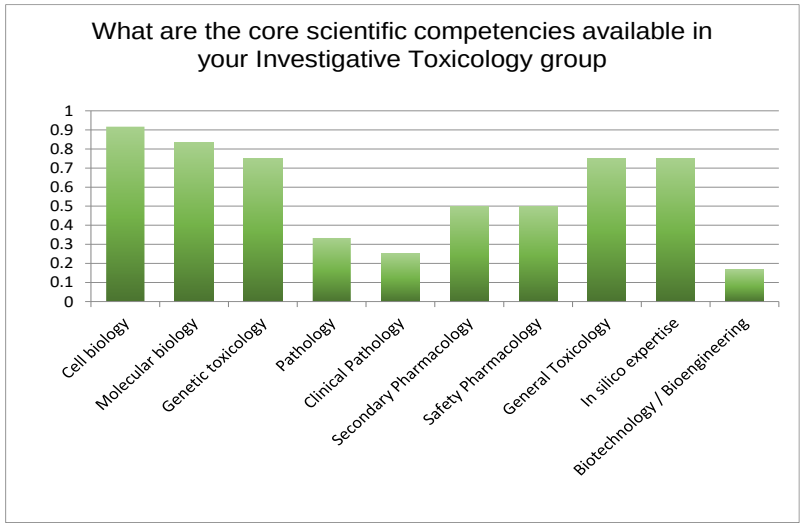

Where are the wet lab based activities of Investigative Toxicology conducted (in %)
Average Number
70
60
50
40
30
20
10
0
within Investigative Toxicology
by other in house groups
CROs
Academia

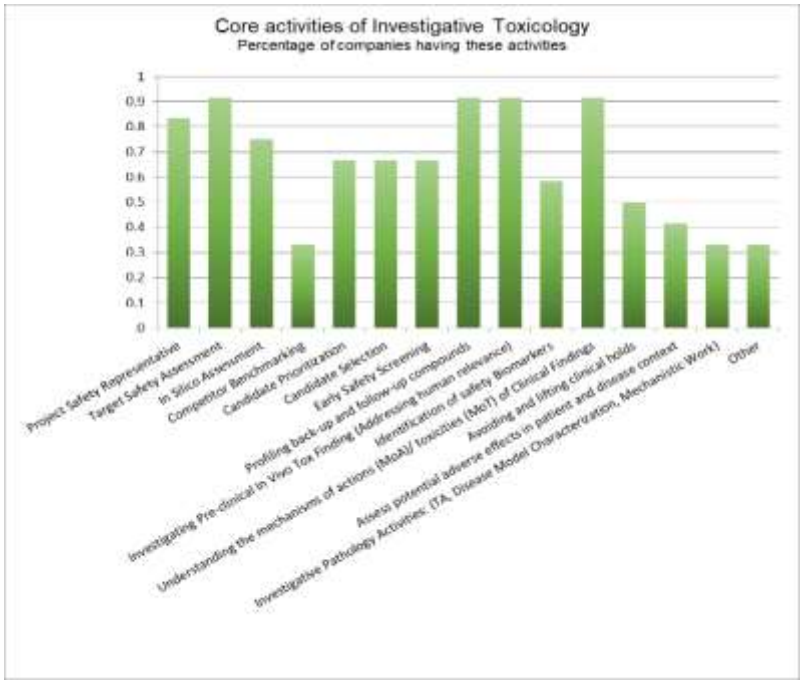

What is the overall academic profile of your Investigative Toxicology group as a % of the overall group size
60
50
40
30
20
10
0
PhD or equivalent
BSc/MSc or equivalent
Technical
Temporary personnel (ie Apprentices, Placement students, PhD students, Post-docs)

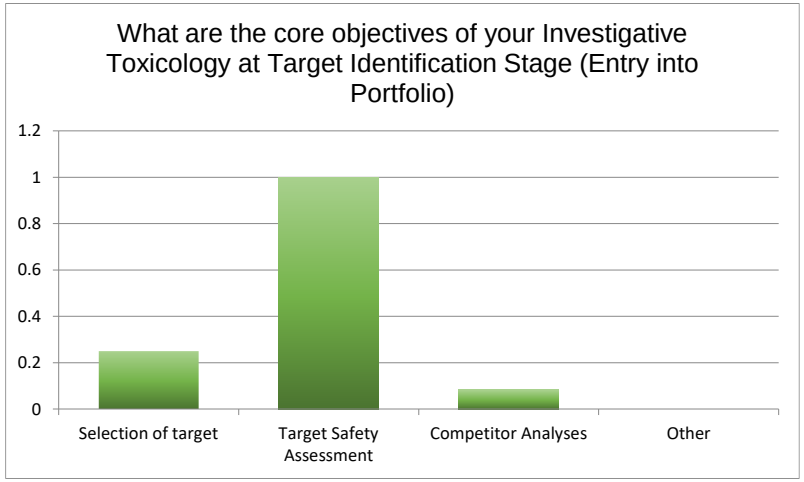

How is the distribution of Investigative Toxicology work (estimate of % of total annual spending) that is conducted under GLP, non-GLP, other QA procedures
Average Number
100
90
80
70
60
50
40
30
20
10
0
GLP
non-GLP
other (e.g. Good Research Practices)

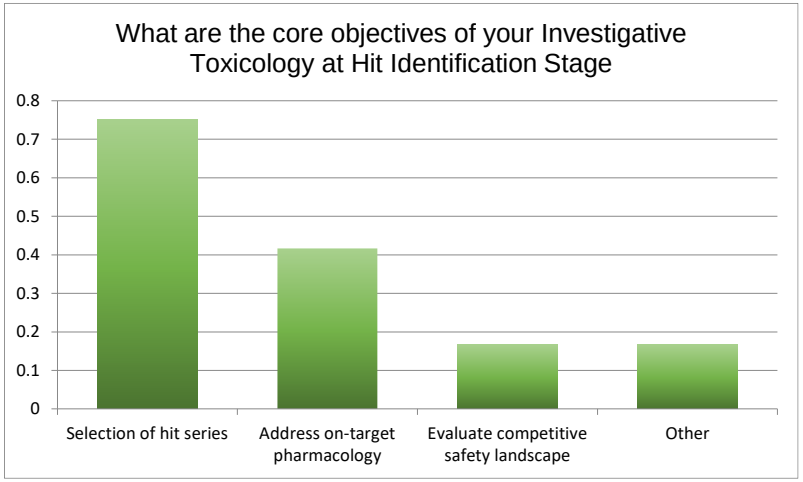

How is the budget distribution of Investigative Toxicology activities performed in house vs externally (%)
Average Number
80
70
60
50
40
30
20
10
0
Percentage in house (i.e., within the company)
Percentage externally (eg, Academia, CROs, consortia)

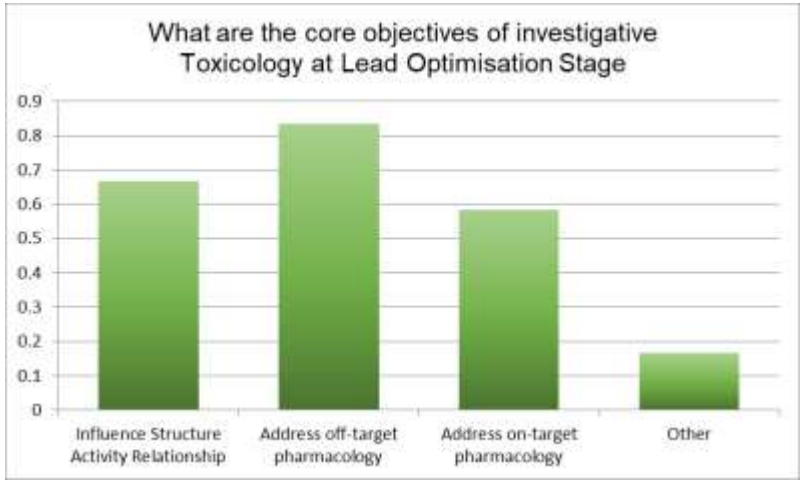

What is the percentage of FTEs' time dedicated to non-project related to innovation versus project support
0.6
0.5
0.4
0.3
0.2
0.1
0
<10%
10-20%
20-30%
30-40%
40-50%
>50%

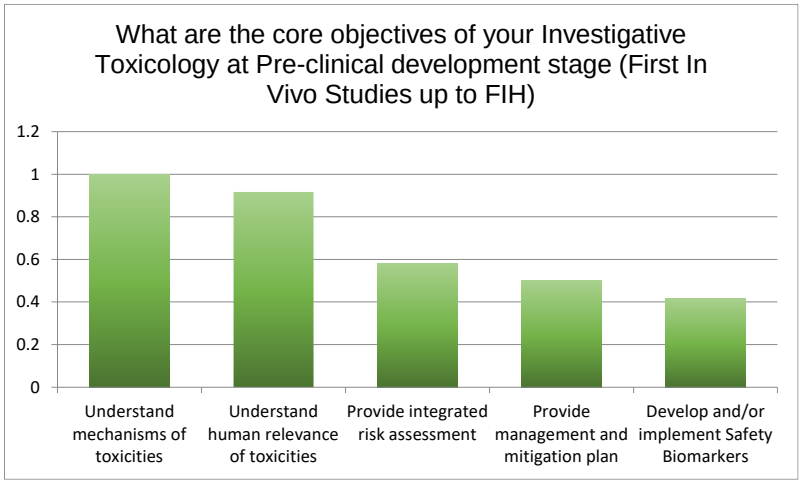

What are the core scientific competencies available in your Investigative Toxicology group
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Cell biology
Molecular biology
Genetic toxicology
Pathology
Clinical Pathology
Secondary Pharmacology
Safety Pharmacology
General Toxicology
In silico expertise
Biotechnology / Bioengineering

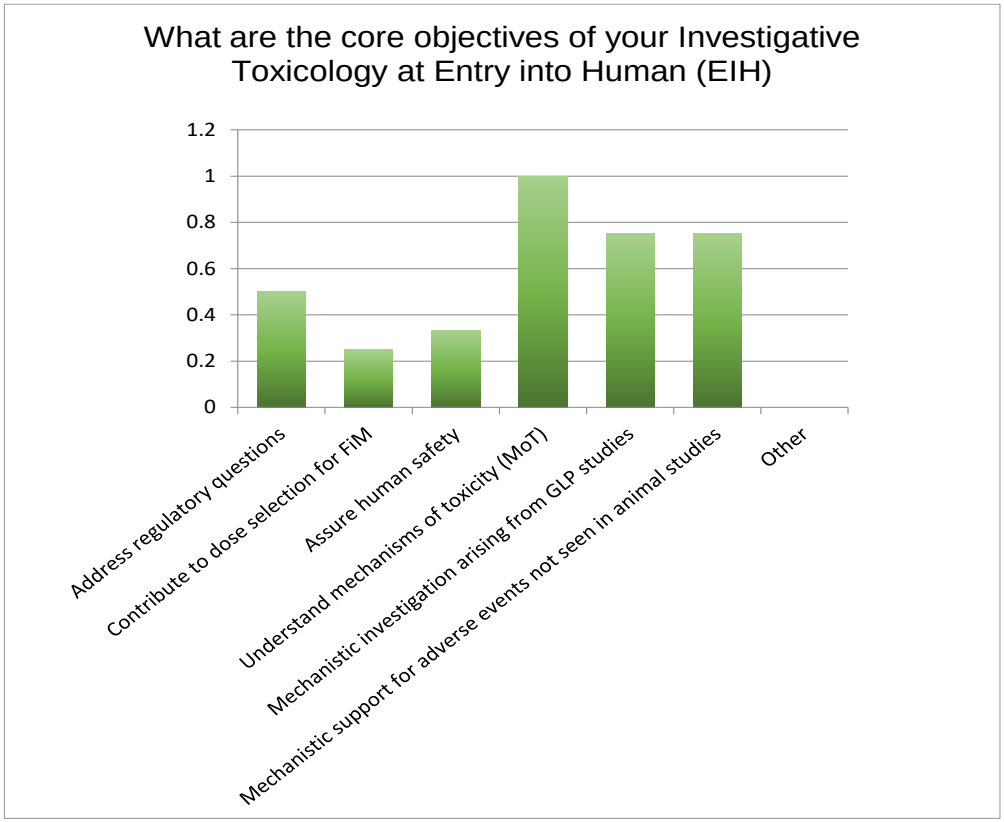

Core activities of Investigative Toxicology
Percentage of companies having these activities
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Project Safety Representative
Target Safety Assessment
In Silico Assessment
Competitor Benchmarking
Candidate Prioritization
Candidate Selection
Early Safety Screening
Profiling back-up and follow-up compounds
Investigating Pre-clinical in Vivo Tox Finding (Addressing human relevance)
Understanding the mechanisms of actions (MoA)/ toxicities (MoT) of Clinical Findings
Identification of safety Biomarkers
Assisting and lifting clinical holds
Assess potential adverse effects in patient and disease context
Investigative Pathology Activities: (TA, Disease Model Characterization, Mechanistic Work)
Other

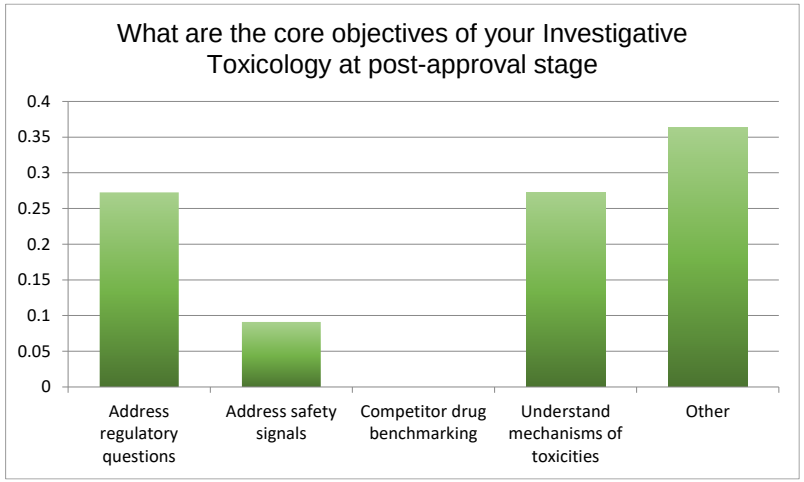

What are the core objectives of your Investigative Toxicology at Target Identification Stage (Entry into Portfolio)
1.2
1
0.8
0.6
0.4
0.2
0
Selection of target
Target Safety Assessment
Competitor Analyses
Other

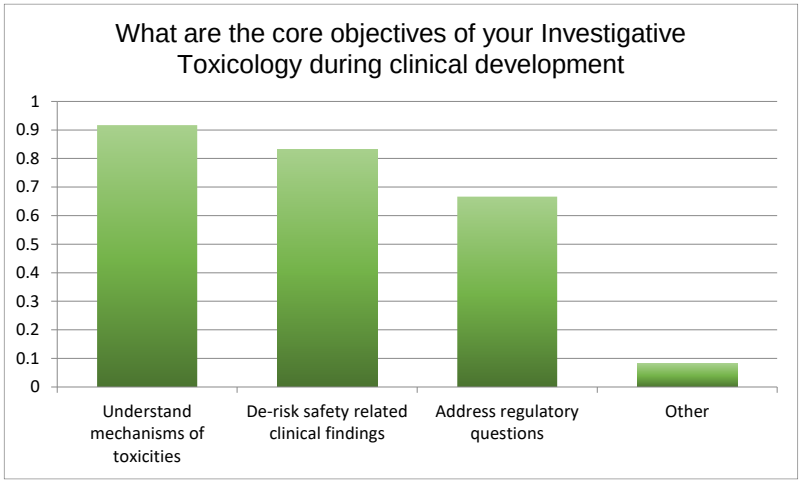

What are the core objectives of your Investigative Toxicology at Hit Identification Stage
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Selection of hit series
Address on-target pharmacology
Evaluate competitive safety landscape
Other

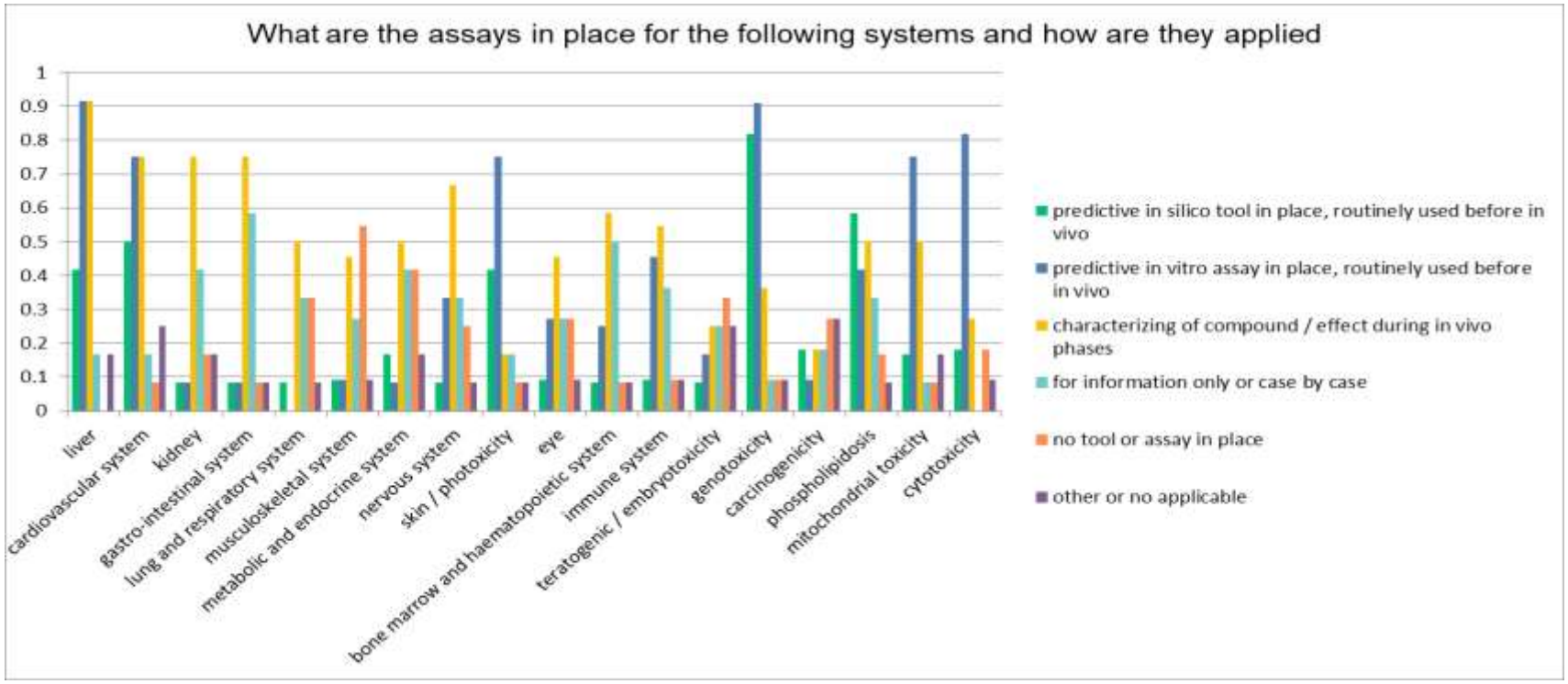

What are the core objectives of investigative Toxicology at Lead Optimisation Stage
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Influence Structure Activity Relationship
Address off-target pharmacology
Address on-target pharmacology
Other

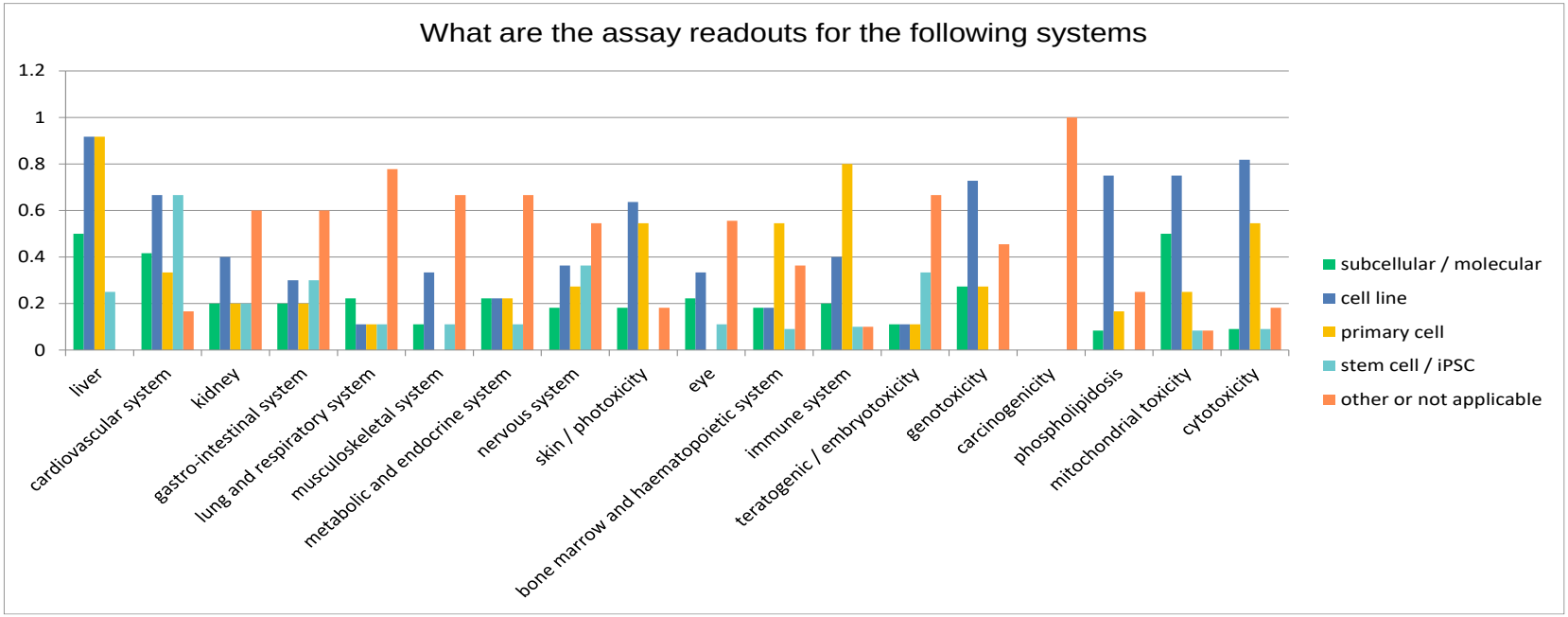

What are the core objectives of your Investigative Toxicology at Pre-clinical development stage (First In Vivo Studies up to FIH)
1.2
1
0.8
0.6
0.4
0.2
0
Understand mechanisms of toxicities
Understand human relevance of toxicities
Provide integrated risk assessment
Provide management and mitigation plan
Develop and/or implement Safety Biomarkers

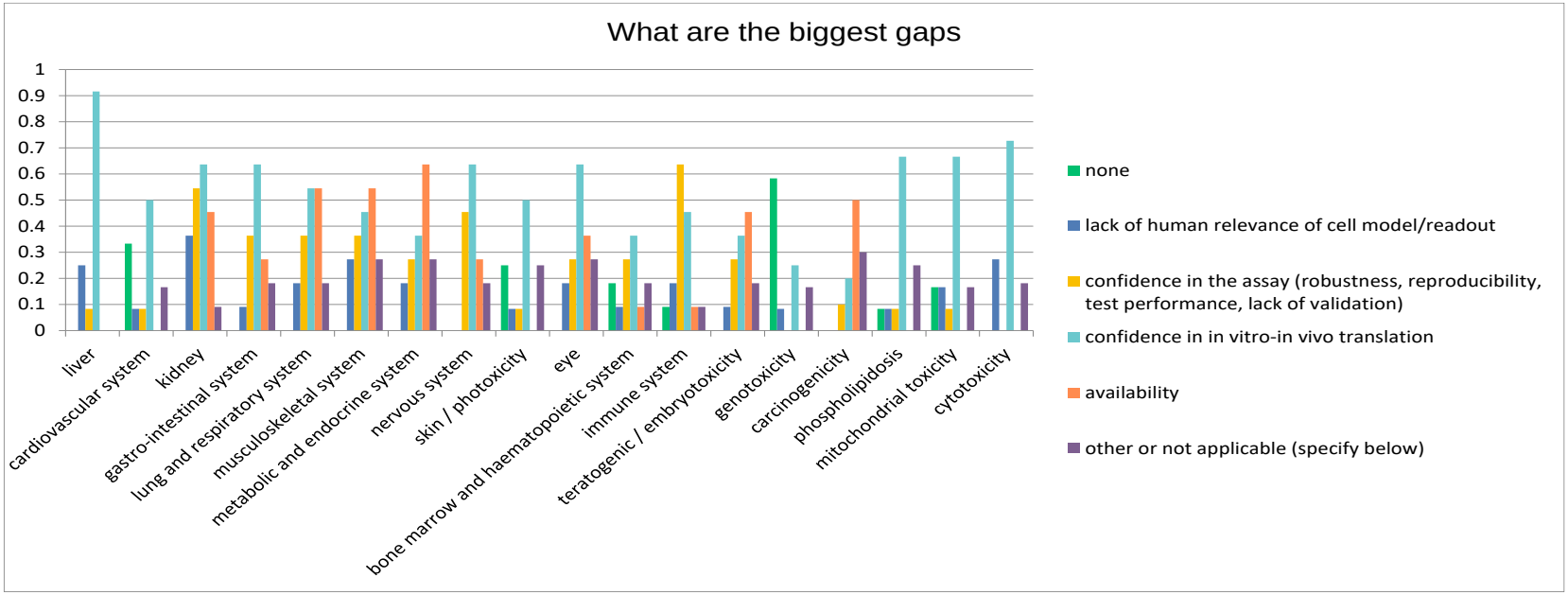

What are the core objectives of your Investigative Toxicology at Entry into Human (EIH)
1.2
1
0.8
0.6
0.4
0.2
0
Address regulatory questions
Contribute to dose selection for FiM
Assure human safety
Understand mechanisms of toxicity (MoT)
Mechanistic investigation arising from GLP studies
Mechanistic support for adverse events not seen in animal studies
Other

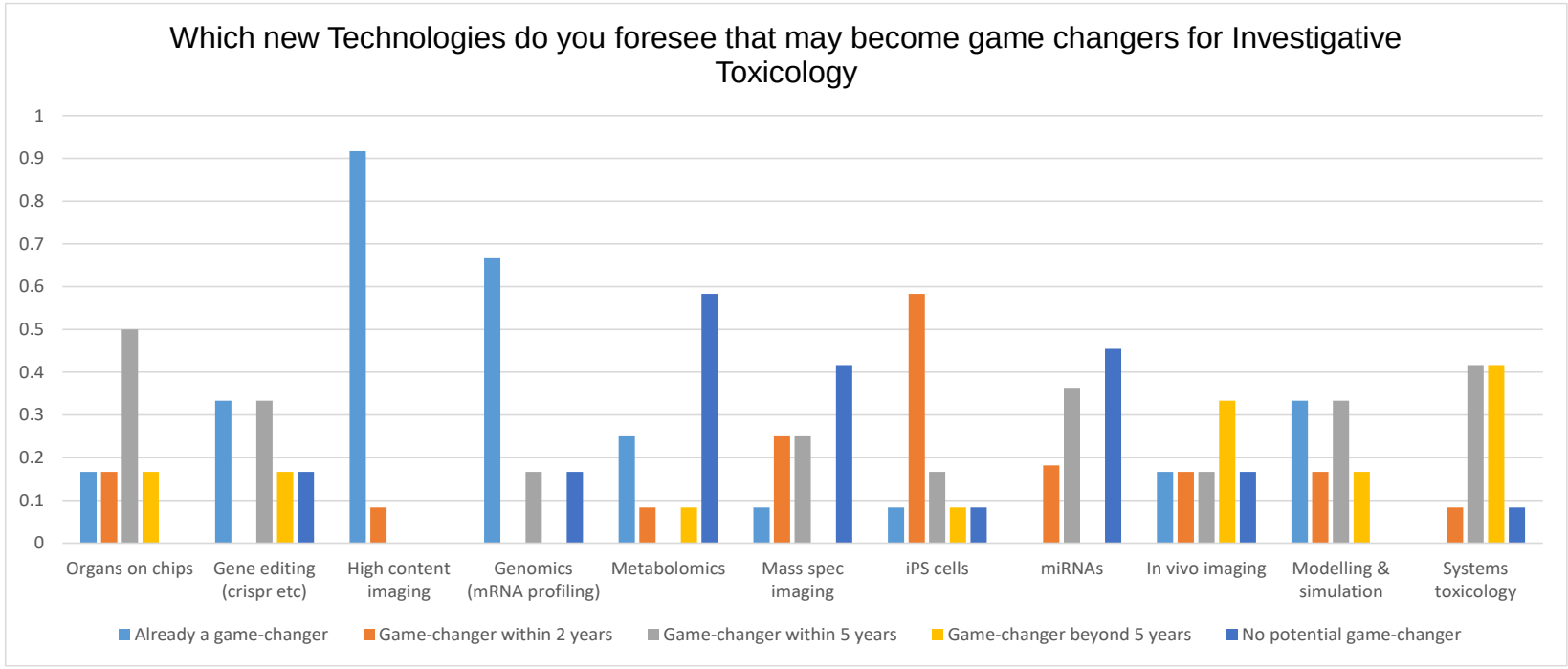

What are the core objectives of your Investigative Toxicology at post-approval stage
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Address regulatory questions
Address safety signals
Competitor drug benchmarking
Understand mechanisms of toxicities
Other

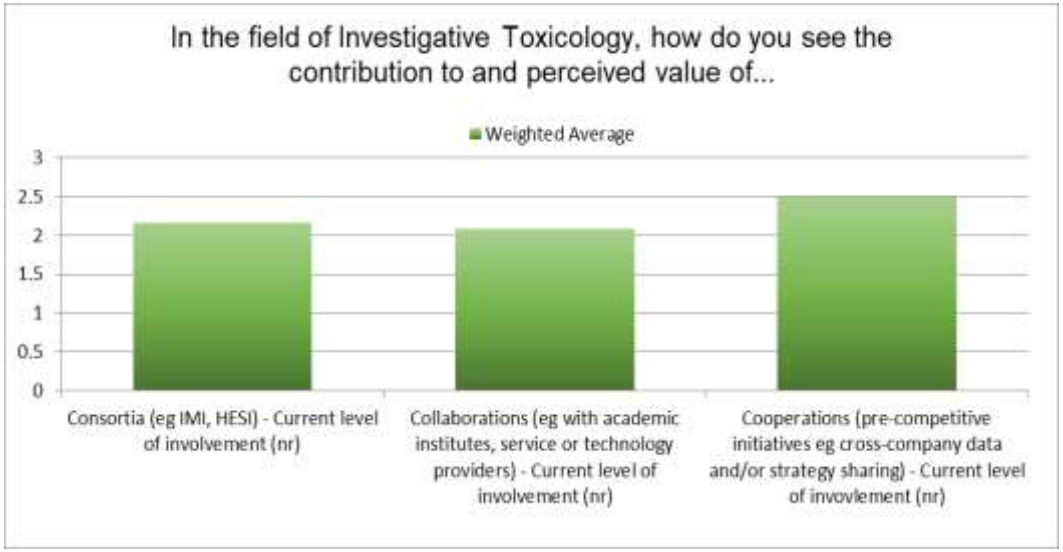

What are the core objectives of your Investigative Toxicology during clinical development
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Understand mechanisms of toxicities
De-risk safety related clinical findings
Address regulatory questions
Other

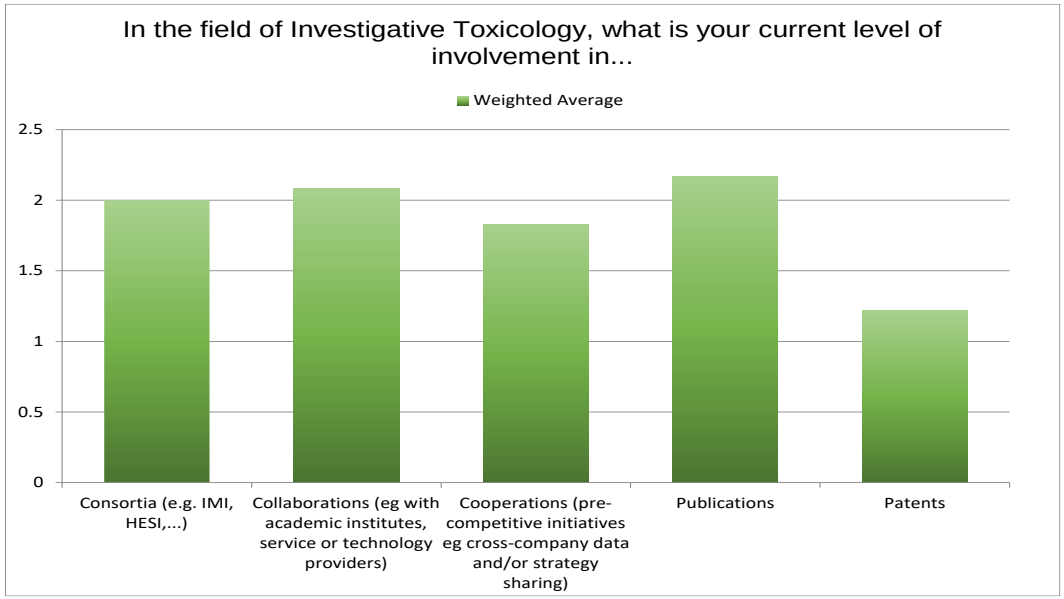

What are the assays in place for the following systems and how are they applied
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
liver
cardiovascular system
kidney
gastro-intestinal system
lung and respiratory system
musculoskeletal system
metabolic and endocrine system
nervous system
skin / photoxicity
eye
bone marrow and haematopoietic system
immune system
teratogenic / embryotoxicity
genotoxicity
carcinogenicity
phospholipidosis
mitochondrial toxicity
cytotoxicity
predictive in silico tool in place, routinely used before in vivo
predictive in vitro assay in place, routinely used before in vivo
characterizing of compound / effect during in vivo phases
for information only or case by case
no tool or assay in place
other or no applicable


What are the assay readouts for the following systems
1.2
1
0.8
0.6
0.4
0.2
0
liver
cardiovascular system
kidney
gastro-intestinal system
lung and respiratory system
musculoskeletal system
metabolic and endocrine system
nervous system
skin / photoxicity
eye
bone marrow and haematopoietic system
immune system
teratogenic / embryotoxicity
genotoxicity
carcinogenicity
phospholipidosis
mitochondrial toxicity
cytotoxicity
subcellular / molecular
cell line
primary cell
stem cell / iPSC
other or not applicable


What are the biggest gaps
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
liver
cardiovascular system
kidney
gastro-intestinal system
lung and respiratory system
musculoskeletal system
metabolic and endocrine system
nervous system
skin / photoxicity
eye
bone marrow and haematopoietic system
immune system
teratogenic / embryotoxicity
genotoxicity
carcinogenicity
phospholipidosis
mitochondrial toxicity
cytotoxicity
none
lack of human relevance of cell model/readout
confidence in the assay (robustness, reproducibility, test performance, lack of validation)
confidence in in vitro-in vivo translation
availability
other or not applicable (specify below)


Which new Technologies do you foresee that may become game changers for Investigative Toxicology
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Organs on chips
Gene editing (crispr etc)
High content imaging
Genomics (mRNA profiling)
Metabolomics
Mass spec imaging
iPS cells
miRNAs
In vivo imaging
Modelling & simulation
Systems toxicology
Already a game-changer
Game-changer within 2 years
Game-changer within 5 years
Game-changer beyond 5 years
No potential game-changer


In the field of Investigative Toxicology, how do you see the contribution to and perceived value of...
Weighted Average
3
2.5
2
1.5
1
0.5
0
Consortia (eg IMI, HESI) - Current level of involvement (nr)
Collaborations (eg with academic institutes, service or technology providers) - Current level of involvement (nr)
Cooperations (pre-competitive initiatives eg cross-company data and/or strategy sharing) - Current level of involvement (nr)


In the field of Investigative Toxicology, what is your current level of involvement in...
Weighted Average
2.5
2
1.5
1
0.5
0
Consortia (e.g. IMI, HESI,...)
Collaborations (eg with academic institutes, service or technology providers)
Cooperations (pre-competitive initiatives eg cross-company data and/or strategy sharing)
Publications
Patents

Supplementary Table 1 **| Review of in vitro cellular assays available for investigative toxicology**

| Organ | Cell lines | Primary cells | iPS | 3D/organoids | MPS |
|---|---|---|---|---|---|
| CV | Mouse HL-1 cells: atrial phenotype, spontaneously beat following pharmacological stimulation<br>Human AC16: immature electrophysiology possible, not extensively used.<br>Rat myoblast H9c2 cells: skeletal muscle phenotype but unresponsive to electrical stimulation.<br>**Key strengths:** amenable to plate-based assays, cost-efficient<br>**Key limitations:** proliferative, limited cardiomyocyte phenotypes both morphologically and functionally, poor predictive value for cardiotoxicity assessment.<br>**Refs:** [1-3] | Primary native cardiac cells (cardiomyocytes, fibroblasts, endothelial cells) from different species (rodent, non-rodent human) can be isolated, evaluated and used for testing.<br>**Key strengths:** Physiology similar to *in vivo* situation; different cell types, degrees of maturation, phenotypes, pathophysiological conditions can be obtained.<br>**Key limitations:** No longer spontaneously beating. Reproducibility between sources of cells. Availability for certain species (e.g. Human). Ethical and 3Rs considerations. Limited compatibility for screening purposes.<br>**Future opportunities:** Improve tissue availability, cells isolation and preservation technique to make them compatible with screening<br>**Refs:** [4,5] | Mouse and human stem cell derived cardiomyocytes<br>**Key strengths:** amenable to long term culture and plate-based assays, replicate pharmacological effects, terminally differentiated, respond to electrical stimulation, applicable to screening in early discovery. Biomarker assessment<br>**Key limitations:** immature phenotype in terms of morphology and function (disorganized sarcomeres, incorrect calcium handling, unphysiological electrophysiology), mixed cardiomyocyte populations, expensive.<br>**Future opportunities:** development of patient derived cardiomyocytes mimicking disease or toxic pathophysiology<br>**Refs:** [6,7] | Human iPS cardiomyocyte only and multi-cell microtissues<br>**Key strengths:** viable and functional for weeks, pharmacological responses represent intact heart, non-cardiomyocytes able to be integrated, applicable to screening in early discovery, emerging as a predictive model<br>**Key limitations:** physiologically and morphologically immature, bespoke methods required for endpoint assays Lacking neuronal and humoral control<br>**Future opportunities:** simultaneous assessment of cardiac function and structure, incorporation of patient derived iPS cell types<br>**Refs:** [7-9] | Human iPSC-based cardiac MPS, incorporating elements of mechanical and electrical loading, non-cardiomyocytes and perfusion systems.<br>**Key strengths**: Maturing morphological and functional phenotype (positive force frequency relationship, physiological resting membrane potential, aligned sarcomeres), incorporation of additional cell types<br>**Key limitations**: pharmacology predictive value largely to be determined, expensive, limited throughput<br>**Future opportunities:** incorporation of larger array of non-cardiomyocytes, pharmacological validation and predictive value to simultaneously assess cardiac function and structure overtime, incorporation of other organ systems e.g. autonomic nervous system and renal control, incorporation of cardiac pre and after load, 3Rs impact<br>**Refs:** [9-12] |
| Liver | Human hepatoma cell line Hep G2 is commonly used as a first screen for identification and discarding of toxic compounds and compound series and ranking within chemical series.<br>**Key strengths:** low cost and possibility for high throughput.<br>**Key limitations:** A number of cell-lines have limited functionality, low metabolism, endpoints assess cell health or a specific organelle function, not organ toxicity.<br>**Future opportunities**: HepaRG cell line presently offers a model with hepatocyte functionality and reproducible phenotype e.g. | Animal or human source, most 2D models are applicable for a short-term culture as hepatocyte phenotype is rapidly lost.<br>**Key strengths:** simple, suitable for short-term culture assays for HTS, can test for phenotypic differences and response to compounds metabolism and cytotoxicity's<br>**Key limitations**: Donor-to donor variation, endpoints mainly assess cytotoxicity or specific organelle function and variable quality of primary hepatocytes<br>**Ref :** [17] | Stem cell derived hepatocyte-like cell (SC-HLC) lines, also commercially available.<br>**Key limitations**: Robust differentiation protocols not yet available and SC-HCLs do not possess human adult hepatocyte phenotype. Adequate benchmarking to physiologically relevant (e.g. freshly isolated hepatocyte) and non-relevant (e.g. HepG2) is lacking.<br>**Future opportunities**: SC-HLCs have potential, when adequately mature to provide a limitless supply of human liver-like cells for HTS and for more | Microtissue and co-culture models of cell lines and primary human hepatocytes without or with nonparenchymal cells, (incl. liver slices)<br>**Key strengths:** Liver cell polarity and phenotype, in coculture models, tissue/cell functionality can be maintained for several weeks. May offer a model for detection of (some) chronic and inflammation mediated toxicity. Increased sensitivity to liver toxins vs. 2D.<br>**Key limitations:** Higher cost, relatively easy to handle with respect to other models but lower throughput. | Microphysiological *in vitro* systems in which the physiological and pharmacological characteristics resemble more closely native tissues (30-32).<br>**Key limitations:** Not fully characterised and currently only limited data to suggest improvements in detection and sensitivity compared to 3D/organoids to detect chemical insult on the hepatocyte. At present, systems do not fully integrate the immune functionality vasculature and bile flow and bile collecting ducts.<br>**Future opportunities**: These platforms are intended to represent a significant step |

| | | | | | |
|---|---|---|---|---|---|
| | metabolic capacity, bile acid production and transporter proteins for primary screening **Refs**.[13 14 15 16] | | complex models (3D, co-culture, MPS) with minor batch variability, and to enable to test individual-specific toxicity. **Ref:** [18] | **Refs :** [19, 14, 20] | forward towards the development of models that faithfully reflect native tissue response to compound toxicities in human. The stability of culture models (organ-on-chip, MPS) may permit more long-term experiments, physiologically, pharmacologically and toxicologically relevant to human liver. **Refs:** [21,22] |
| Lung | Alveolar epithelial type II cell (AECII): A549 cell line generated from human adenocarcinoma, exhibiting features of alveolar type II cells. **Key strength:** building epithelial layer when cultivated at air-liquid interphase<br>Bronchoepithelial cell lines: generated by virus-dependant immortalization of normal human bronchial epithelial cells (BEAS-2B cell line, adenovirus) or human tracheal (16HBE14o- cell line, SV40)<br>**Key strength:** Formation of tight epithelium or bronchial epithelial morphology allows high throughput testing of epithelium damage<br>Alveolar macrophage: NR8383 cell line established from Sprague-Dawley rats, maintain a large number of macrophage characteristics of inflammatory response and are widely used as *in vitro* system to assess cellular response to drug candidates (e.g. phospholipidosis, cytokine release) where inhalation is intended route of delivery<br>**Key strength of all cell lines:** Established tools for toxicity testing in high throughput screenings and for mechanistic investigations. Low cost, unlimited source, easy handling | Normal human tracheobronchial epithelial cells or disease biopsy material used in cytotoxicity, function and barrier resistance assays.<br>Normal human tracheobronchial epithelial cells grown at air-liquid interface can differentiate to form mucocilliary morphology. Have been used *in vitro* to measure response to xenobiotics. Display CYP2A activity and CYP1A1/1B1 inducibility and activity, relevant for metabolic bioactivation toxicity.<br>**Ref**: [26] | Several differentiation protocols for producing lung epithelial cells from iPSC have been published. Further optimisation and testing of protocols is required<br>**Ref**: [27] | 3D models of airway epithelium have been developed by cultivating primary airway epithelial cells on porous membranes at the air-liquid interface with the basolateral surface bathed in culture media and the apical side exposed to humidified air, leading to morphologically and functionally differentiated systems: e.g. MucilAir and EpiAirway models<br>**Key strength: G**ood representation of bronchial epithelium, including cilia function and mucus production. Air exposed 3D cultures allow the toxicity of air borne particles to be studied in a manner that closely mimics inhalation *in vivo*. Further testing is needed<br>**Key limitations:** Limited number of donors for model generation, high costs<br>**Future opportunities:** Have potential as integral part of multi-organ chips in microphysiological systems<br>**Ref:**[28] | Lung on a chip models emerged form microphysiological system approaches of several companies. E.g. Wyss Institute, developed a biomimetic microdevice with physiological breathing movements. Proof of principle for mechanistic toxicity and disease testing achieved in collaborations with industry<br>**Key strength:** Advancing *in vitro* models with complex physiologic features (e.g. breathing). Proof of principle for mechanistic toxicity and disease testing achieved in collaborations with industry<br>**Key limitations:** Low throughput, laborious handling, control of xenobiotic administration needs further optimization and validation.<br>**Future opportunities:** Further advancement of lung chips may have the potential to partly replace animal experiments with<br>**Ref :**[29] |

| | | | | | |
|---|---|---|---|---|---|
| | **Key limitation of all cell lines:** General genomic issues with cancer cell lines or genetic transformation, less lung cell features compared to primary cells **Refs**: [23-25] | | | | |
| Kidney | Established kidney cell lines (e.g. HEK293) not broadly used for safety assessment. As drug-induced AEs in kidney mostly affect tubulus, focus is on cell models representing renal cortex. Relevant cellular models include e.g. TERT immortalized proximal tubular cells (RPTEC-TERT1). **Key strength**: Immortalized cell lines expressing kidney transporters are used to support mechanistic understanding of toxicity **Key limitations**: Not able to model the complexity of kidney physiology, some key transporters are not expressed **Ref**: [30] | Primary cells from kidney available (PTEC, podocytes, mesangial cells). Most established in drug safety are primary proximal tubular cells which can be obtained from various resources and cultured under standard conditions. **Key strength**: human origin applied for mechanistic understanding using human biomarkers **Key limitations**: Limited phenotypic stability. Source of human material limited, inter-individual differences may influence results, difficult preparation **Refs**: [31,32] | iPS-derived kidney cells have been reported and show promising predictivity to detected drug-induced nephrotoxicity. Further validation ongoing **Key Strength**: Proximal tubular like IPSC have demonstrated 87% accuracy in detecting nephrotoxicity, can be used to build 3D Models **Key limitations:** Differentiation Protocols often complex **Ref**: [33] | Area is in its infancy, mainly used for pharmacological investigations but there are promising approaches for 3d cultured kidney cells **Key limitations:** so far very limited applications for toxicological application published **Future opportunities:** Organoids that may capture complex architecture of Kidney **Refs**: [34,35] | Several groups working on developing microphysiological, organ on a chip like models recapitulating kidney. Mostly focusing on proximal tubular cells and proper architecture. Bioprinting has been used to create 3D human renal proximal tubules *in vitro* embedded within an extracellular matrix and housed in perfusable tissue chips, maintained for greater than two months. **Key strength:** the presence of fluid shear stress makes cells more sensitive to the drugs, and the use of primary cells makes the *in vitro* renal toxicity testing more physiologically relevant. **Key limitations**: However, high-throughput screening on the kidney chip platform has not yet been achieved. Besides, nephrotoxicity induced by drug metabolites can hardly be studied directly by kidney-on-a-chip. **Future opportunities**: Hyphenation of other organ chips, especially liver chips, is desirable for in-depth study of nephrotoxicity of novel drug candidates. **Refs**:[36,37] |
| CNS | Neuronally differentiated mouse embryonal carcinoma P19 neurons human neuroblastoma SH-SY5Y cells and rat adrenal pheochromocytoma PC12 cells have been used for CNS toxicity assessment. Cytotoxicity, intracellular esterase activity, neurite outgrowth and mitochondrial function endpoints have been used. The importance of including | Simple and rapid multiwell microelectrode array (MEA) technology has been used for the simultaneous determination of test compound effects on spontaneous electrical activity and cell health from networks of primary rat cortical neurones. The mixed neuronal culture consisted of glutamatergic and GABAergic neurons and glia. The neurons contained both | *In vitro* neurotoxicity in a human system based on two different types of human iPSC-derived cells and MEA technology have been described. iPSC-derived peripheral neurons exhibited burst-like activity on MEA chips, consistent with a functional neuronal network. Proof of concept studies indicated sensitivity to neurotoxic drugs | Given the importance of cell-to-cell interactions in the brain, laboratories have begun to develop brain cell culture models. Extracellular matrix scaffolds seeded with primary cortical neurons have also been assessed and human iPSCs differentiated into neural progenitor cells and neuronal astroglia lineages and cultured in 3D aggregates have shown | Brain is perhaps the most complex organ and therefore nervous systems on chip must reflect this complex organisation, multicellular interactions, blood brain barrier compartment and possibly a connection to peripheral and spinal cord MPS. Such system must also be amenable to high content imaging endpoints. Recent advances demonstrate |

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| | astrocytes when predicting acute toxic potential using a neuronal screening has been highlighted by work with pure neuronal (NT2.N) and astrocytic (NT2.A) and integrated neuronal/ astrocytic (NT2.N/A) cell systems derived from the human NT2.D1 cell line.<br>**Key strengths:** low cost, possibility to high throughput, relative constancy of the lines<br>**Key limitations:** Cell lines have limited functionality, proliferate unlike neurons.<br>**Refs**:[38,39] | axons and dendrites and form synapses. The assay measured spontaneous action potential "spikes" and groups of spikes, or bursts.<br>**Key strengths:** Supposedly closer to brain or peripheral cells than cell lines, may express tissue receptors, channels and functions.<br>**Key limitations**: Difficult to acquire, expensive, lack of reproducibility.<br>**Ref**:[40] | and drugs associated with seizure-liability<br>**Key strengths:** Reproducible, easy access, relatively cheap once established. A compromise between primary cells and cell lines. Can be induced in various NS types, e.g., neurons, glia, astrocytes.<br>**Key limitations:** Remain embryonic in nature<br>**Ref**:[41] | promise. Biological and disease characterisations indicate promise.<br>**Key strengths:** Enhances inter-neuronal connections, but also a proper 3D interaction with glial compartments.<br>**Key limitations:** Long to establish, difficult to maintain, low throughput<br>**Refs**:[42] | the feasibility of such complex system for diseases and toxicology studies.<br>**Key strengths:** Fluidics for proper BBB simulation, possible to study interactions with other MPS, immune compartment possible.<br>**Key limitations:** Long to establish, difficult to maintain, complex endpoints, low throughput<br>**Ref**: [43] |
| GI | Caco-2 derived from colorectal adenocarcinoma has been the standard cell line used *in vitro* for GI function and drug absorption.<br>**Key strengths:** Low cost, possibility to high throughput, relative constancy of the lines. Caco2, HT29, T84 cells in investigations of the transport and antioxidant/anti-inflammatory potential. For example, HT29 cell lines mimics both enterocytes and goblet cells<br>**Key limitations:** limited functionality – lack of mucus layer or the interactions between the epithelium and the stroma. Prolonged culture time before use (i.e. 3 weeks for Caco-2)<br>**Future opportunities**: Improved physiological function in novel microfluidic devices<br>**Ref**: [44] | Tissue-derived intestinal epithelial cell cultures support of cytotoxicity, absorption, metabolism. Primary stem cells (SC) are utilized for organoid cultures. Cross-species organoids have been described. A reproducible protocol for maintaining long term primary cell cultures from intestinal tissue is a challenge (Lukovac and Roeselers, 2015)<br>**Key strengths:** SC can differentiate into all intestinal epithelial cells (including also stem and progenitor cells). Cross-species comparisons a reality<br>**Future opportunities:** Inclusion of SC-derived organoids in microfluidics devices<br>**Ref**: [45] | iPSC can be directly differentiated into intestinal-like tissue *in vitro*. Limited data due to success of primary SC application. Knock-out lines being developed (also Caco-2 KO lines)<br>**Key strengths:** Availability and sustainability of lines. Reproducible and relatively cheap.<br>**Ref**: [46] | 3D organoids and multicellular microtissue enable functional comparison across species, GI regions, and healthy versus disease.<br>**Key strengths:** Multi-cellular systems in 3D, allows connection and communication between cells<br>**Refs**: [44,47] | Gut-on-chip models offer potential for pulsatile flow, peristaltic stretch, co-culture with inflammatory cell and/or commensal microbes, and activation of sensory neurons. Significant potential, but very early stages of assessment.<br>**Key strengths:** Intestinal models are very well suited for microfluidic organ-on-a-chip systems.<br>**Key Limitations:** Low throughput and time required to establish models<br>**Future opportunities:** Connecting the different areas of the intestine in one "chip", including engineering in peristalsis and vascularization. Better understand the role of microbiota-derived molecules in modulating the intestines response<br>**Ref**: [48] |

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