Review

Systems-level understanding of toxicology and cardiovascular system

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Abstract: The extensive networks of molecular and functional alterations that take place at many levels of biological structure are studied using traditional toxicology and quantitative research. As society places more emphasis on the potential health concerns connected with exposure to chemicals encountered in daily life, it is imperative that more accurate and predictive risk-assessment tools be developed. The development of such strategies necessitates a comprehensive understanding of the mechanisms by which xenobiotic chemicals injure and impair biological systems, followed by the formulation of mathematical models that quantitatively describe these mechanisms. By figuring out how exposure impacts biological networks, the integrated data analysis enables the creation of mathematical models that predict toxicological processes. The most recent developments in computer analysis, bioanalytical methods, and the potential for more precise risk assessment are all included in this review effort. Human health is seriously threatened by cardiovascular toxicity in both medication development and the environment. Because of the cardiovascular system's considerable physiologic flexibility, variety of presentations, and high prevalence of underlying natural disease, xenobiotic injury can be difficult to detect. As a result, it is essential to comprehend these characteristics completely and employ a thorough evaluation technique. The concept of system-level toxicity has been explained in detail here.

Keywords: Systems toxicology; drug discovery; cardiovascular system; mathematical models; biological networks; data integration

1. Introduction

The primary goal of toxicologists is to investigate and attempt to forecast the detrimental effects of chemicals on biological systems [1–3]. Early in the medication development process, it is very difficult to predict human toxicity due to the complexity of biological systems. In vitro, preclinical, and clinical approaches have been used recently to investigate the effects of chemicals on biological systems [4,5]. The deluge of data from various sources, including omics-level research and data collection, has resulted in an abundance of knowledge about biological systems. This abundance of data has not yet provided accurate toxicity estimates in a single system or the capacity to extrapolate between systems because of a lack of thorough and rigorous data integration. The computer technologies used in the systems biology discipline are now used in a new subject called systems toxicology. tackling toxicity-related issues [5–9].

Systems toxicology integrates quantitative analysis of massive datasets with different levels of complexity, including molecular and functional changes happening at different levels of biological structure, with conventional toxicology methodologies [7,10,11]. A deeper comprehension of the causal relationship between exposure-induced molecular alterations and adverse consequences results from systems toxicology research that focusses on the biological mechanisms and molecular pathways affected by toxicant exposure (**Figure 1**). Systems toxicology aims to decipher the toxicological pattern of active substances that interact with living organisms [12–14]. This field combines computer science, chemistry, toxicology, systems biology, and mathematics. Network models and quantitative assessments of molecular and functional alterations at various levels of biological structure are integrated with conventional toxicological techniques. To characterise and evaluate interactions between potential hazards and the components of a biological system, the multidisciplinary Systems Toxicology approach integrates principles from chemistry, computer science, engineering, mathematics, and physics with high-quality experimental data gathered at the molecular, cellular, organ, organism, and population levels [5,15–17]. Its objective is to give a comprehensive, dynamic, quantitative, and mechanistic understanding of toxicological processes so that complex (emergent) negative effects can be accurately predicted and modelled. The technique lays the groundwork for switching between in-vitro and in-vivo model systems and study contexts (e.g., ecosystem, human).

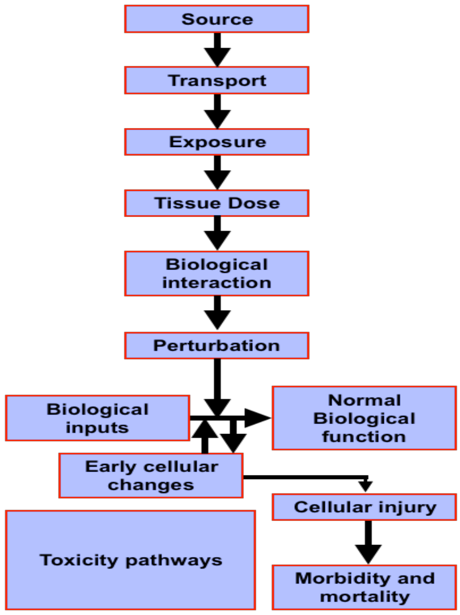

Figure 1. Summarized approach for toxicity study.

Systems Toxicology may contribute to a new paradigm for risk assessment because of its ability to extrapolate from early and highly sensitive measurable molecular and cellular processes to medium- and long-term organism-level outcomes. More precise and predictive risk-assessment techniques have been developed as a result of growing public awareness of the possible health risks associated with exposure to widely used chemicals. To create such remedies, a deep understanding of the mechanisms by which xenobiotic chemicals disrupt biological systems and may be harmful is required. Therefore, Systems Toxicology approaches combine state-of-the-art analytical and computational tools to provide state-of-the-art approaches to obtaining such mechanistic insight. One method for identifying and using biomarkers to improve safety assessments is systems toxicology. In the context of an exposure, systems toxicology quantifies measurable system-wide molecular changes and establishes a causal network of molecular processes that link exposures to harmful outcomes (i.e., functional and apical end goals). The next step will be to create mathematical models that can quantitatively explain these processes. By determining how exposure modifies biological networks, mathematical models that predict the trajectory of toxicological processes can be developed through integrated data analysis [6,9,12,18-24]. This viewpoint takes into account the potential for enhanced computer analysis, risk assessment, and existing bioanalytical processes. Toxicological effects on humans are recognised, evaluated, and avoided in the scientific discipline of toxicology. New drugs must pass stringent testing in preclinical research, clinical trials, and post-marketing studies to make sure the therapeutic benefits outweigh the hazards [2,25–28].

Systems toxicology combines traditional toxicology with quantitative analysis of vast networks of molecular and functional changes at different levels of biological structure. More precise and predictive risk-assessment techniques have been developed as a result of growing public awareness of the possible health risks associated with exposure to widely used chemicals. To develop such methods, a thorough mechanistic knowledge of how xenobiotic substances interfere with biological systems and cause unwanted effects is required. Therefore, systems toxicology techniques offer modern approaches for obtaining such mechanistic insights by combining state-of-the-art analytical and computational technologies [7,11,29-32]. One method for identifying and using biomarkers to improve safety assessments is systems toxicology. Systems toxicology measures quantified system-wide molecular changes in the context of an exposure (functional and apical end points) and examines a causal chain of molecular processes linking exposures to adverse consequences. These processes are then statistically characterised through the development of mathematical models. Building mathematical models that forecast toxicological processes is made possible by the thorough data analysis that identifies how exposure disrupts biological networks [7,12,22,33,34]. This perspective takes into account current bioanalytical tools, computer analysis, and the possibility of improved risk assessment [6,7,35].

Cardiovascular toxicity (CV) is a major public health concern in medication development and the environment. Because of the CV system's considerable physiologic flexibility, presentational heterogeneity, and high prevalence of underlying natural disease, xenobiotic damage may be difficult to detect. As a result, having a solid grasp of these characteristics and a rigorous evaluation method are essential. While the pathogeneses and symptoms of an increasing number of cardiac and vascular toxins have been identified, their mechanisms of action are usually unknown. The goal of this review was to provide a comprehensive overview of the selected topic. In order to gain a comprehensive and up-to-date understanding of specific toxic injuries, it is recommended that additional research be done in addition to continuously improving methods for assessing them [11,36–38].

Possible Systems Toxicity and Systems Biology research, along with sources for relevant data and methodologies, have been the focus of this review. We have discussed the basic techniques and their potential applications to chemical safety assessments. This comprehensive examination of toxicological data is expected to greatly improve our knowledge of and capacity to forecast the harmful consequences of chemicals [4–

7,9,15,39,40].

2. Toxicology

Figure 1 illustrates our streamlined approach to toxicity testing. The science of toxicology examines the effects of harmful substances or physical elements on living organisms. The cellular, metabolic, and molecular processes that underlie chemical toxicity are studied by toxicologists. Negative consequences might manifest in a number of ways, ranging from sudden death to gradual alterations that take months or years to manifest. At every level of the organism, they can affect a particular organ, cell type, or biochemistry. Medical science has improved its understanding of the effects of dangerous drugs on the body [1,11,12,33,41-47]. It is recognised that previously undiscovered alterations in particular biochemicals in the body can induce various apparent changes in anatomy or bodily functioning. Although the terms toxicant, toxin, and poison are frequently used interchangeably in the literature, there are a few significant distinctions to be made between them. Toxic chemicals can have either general or organ-specific effects. A systemic toxin is a poison that impacts the entire body or many organs. For example, almost all of the body's cells and organs are unable to use oxygen when exposed to the systemic toxin potassium cyanide. Certain organs or tissues may be the target of toxins while other body parts are left unaffected [8,48–51]. These specific regions are known as target organs or target tissues. Here are several examples: Because it damages the tissues that make blood, benzoene is a special kind of organ toxin. Lead affects only three organs in the body—the kidney, the haematopoietic system, and the central nervous system—despite being an organ toxin as well. A hazardous agent is something that puts living things in danger. There may be biological, physical, or chemical processes at play. Toxic substances can be physical (like snake venom), chemical (like cyanide), or biological (like germs). To put it simply, a material with undesired qualities is considered dangerous. It could be a group of toxins or just one toxin. Lead chromate, petrol and asbestos are just a handful of the dangerous materials. In and of itself, lead chromate is a dangerous material [2,4,38,52].

A dangerous material, asbestos is composed of many fibres and minerals whose chemical makeup is unknown. Because petrol is a mixture of numerous components, it is a hazardous material rather than a dangerous chemical. A specific tissue type found in several organs (like connective tissue) may be affected by a toxin. Thus, the term "target tissue" refers to the dangerous location. The body's cells are diverse in size and shape, and they can be grouped based on the kind of tissue and their fundamental structure (e.g., hepatocytes of the liver). Ovum and sperm are examples of cells that are still in the embryonic stage; somatic system cells are the body's non-reproductive cells; and germ cells are the reproductive cells that have the capacity to create new life. They have a single pair of chromosomes for each sex. The germ cells of males and females differentiate into sperm and eggs, respectively. Toxicology to germ cells may have detrimental effects on a developing kid (e.g., birth abnormalities, abortions). Except for reproductive germ cells, every bodily cell is a somatic cell. They possess two sets (or pairs) of chromosomes. A person exposed to somatic cell toxicity may experience dermatitis, malignancy, and mortality, among other issues [11,53-58].

The dose-response relationship is one of the factors that should be considered when assessing the harmful effects of hazardous substances. A dosage is defined as the total amount of a substance that is given all at once. To explain xenobiotic exposure, however, other components are required. The most important factors are the frequency, duration, and overall amount of dosages. Exposure dose, absorbed dose, administered dose, and total dose are among the several forms of dosages. In toxicology, one of the most crucial and fundamental ideas is the dose-response connection. It establishes a link between exposures and various detrimental outcomes. The stronger the reaction, the more harmful the dose. The dose-response connection is supported by data from cell research, human clinical trials, and animal studies [1,5,18,22,41,59,60]. By demonstrating that the chemical was the cause of the observed symptoms, the dose-response relationship establishes causality. the threshold effect, which determines the dose response slope, the lowest dose at which an induced effect occurs, and the rate at which harm accumulates. The sigmoid is the most common type of dose-response curve. A smooth curve that gets as near to the individual data points as is practical is used. For the great majority of effects, small doses are safe. The dose at which toxicity first appears is known as the threshold dosage level. As the dose level increases, the slope gets steeper [1,41,61-66]. Although there is a traditional correlation between dose and reaction or impact, certain medications have a limit beyond which they have no effect. The first is the host's reaction, and the second is the exposure circumstances.

Toxicology studies the effects of harmful substances on living organisms. Toxicology can be found in a variety of places, such as: I Investigating the ways in which poisons harm living things is the focus of mechanistic toxicology. (ii) Descriptive toxicity: To collect data that can be utilised to assess the risk that chemical exposure presents to humans and the environment, descriptive toxicologists conduct toxicity studies. (iii) Regulatory toxicity: A regulatory toxicologist determines if a medicine or other chemical poses a risk that is sufficiently minimal to justify its use for the intended purpose. Among the techniques used to study toxicity are: I in-silico biological experiments that use computer models based on data from previous studies. (ii) In-vitro: uses a model to evaluate potential genetic and biochemical consequences (DNA interactions, bacteria, organisms cultivated in animal cells). In order to ascertain whether they have the capacity to cause cancer or other diseases of interest (iii) in-vivo, experimental animals may be given high dosages throughout their lives [5,13,18,67-71].

Table 1. Methods and tools for toxicity study
The fixed dose procedure (FDP): FDP is used to assess the nonlethal toxicity rather than the lethal dose.
The acute toxic category (ATC) method: It is a sequential procedure in which three animals of the same sex are used in each step.
The up-and-down (UDP) method: The staircase design is another name for the UDP testing strategy. Since this method uses less vertebrate animals in research, it is the toxicological testing strategy that is most frequently advised by different regulatory organizations. The UDP screening procedure entails sequentially treating one animal every 48 hours. For UDP testing, mice that are female are preferred.
Acute toxicity testing for inhalation
Acute toxicity testing for topical preparations
Skin sensitization tests
Repeated dose toxicity testing
Mutagenicity testing
Subchronic oral toxicity testing
Chronic oral toxicity testing
Carcinogenicity testing
One-generation reproduction toxicity testing
Two-generation reproduction toxicity studies
Toxicokinetics
Neurotoxicity studies
Developmental toxicity/embryotoxicity studies
Genetic toxicity testing
SAR (structure activity relationship) analysis
CASE/multi-CASE
TOPKAT
DEREK
ONCOLOGIC
High-throughput methods (Microarray, NGS, etc.,)

3. Computational models

A chemical's toxicity must be determined in order to determine its detrimental effects on humans, animals, plants, and the environment. It's also one of the most important stages of a drug's development. Animal models have long been employed in toxicity testing. However, in vivo animal testing is limited by time, ethical concerns, and budgetary constraints. To have a better understanding of the toxicity process, computational models are employed. The model essentially identifies each molecular component required to replicate toxicity both in vitro and in vivo. Computational toxicology manages and finds patterns and linkages in vast volumes of chemical and biological data by utilising modern computation. High-information-content data streams (such those from microarrays or in vitro high-throughput screening methods), innovative biostatistical methodologies, and the processing capability to analyse these data are all used in computational toxicology. Determining the relative importance of different network components is a similar subject in each biological setting [12,72-79]. Additionally, Table 1[80–83] contains the list of cytotoxicity texting techniques as well as a helpful tool.

Tools are necessary to comprehend the risks and hazards that chemicals, novel materials, and the environment present. Furthermore, models for understanding global gene regulatory networks must be developed in order to better understand how chemicals impact reproduction, gene expression, and blood hormone levels (testosterone) [14,84-88]. Previous research has also investigated the use of high-dimensional gene expression data to reverse engineer complicated interaction networks in order to investigate drugs from a variety of perspectives [89–92]. The study of how medications and other chemicals affect important organs and biological pathways has been made easier by the development of global gene regulatory networks. To sum up, effective systems toxicological knowledge and interpretation require a dynamic multiscale biological model, a potentially causal computable biological network model, and a dynamic negative consequence pathway model. Numerous field-based approaches, including big data analysis, computational chemistry, complex systems modelling, and advanced biological technologies, have been combined at various levels (e.g., Next Generation Sequencing (NGS), imaging, and scanning).

3.1. Biological networks: "Network biology" is a branch of biology that focusses on measuring the networks that underpin many biological systems. From the cell to the Internet, the coordinated action of several constituents that communicate with each other through pairwise interactions determines the behaviour of most large systems. At a high level of abstraction, the components can be boiled down to a collection of nodes joined by links that represent the interactions between two distinct components. A network, or graph in more formal mathematical terminology, is made up of the nodes and linkages. It is challenging to distinguish between the different cellular networks. Physical relationships between molecules, such as interactions between proteins and metabolites, proteins and nucleic acids, and other proteins, are easily recognised thanks to the node-link terminology [93–102]. However, this model can account for more intricate functional relationships. Small molecule substrates, for example, could be viewed as nodes in a metabolic network, where links indicate enzyme-catalyzed reactions that transform one metabolite into another. By combining bioinformatics with the expanding amount of biological and chemical data, network biology has emerged as a promising approach in this field. This method can be used to assess the safety of substances at several levels of human health, including molecular, cellular, and system levels. Network biology can be applied at different levels of complexity. Co-occurrence of diseases can affect the underlying network biology of shared and multifunctional genes and pathways. Comorbidities facilitate an understanding of the impact of external factors such as lifestyle, diet, and patient treatment [93,103-107]. As more and more health transaction data is being gathered electronically worldwide, disease co-occurrences are beginning to be quantified. A solid basis for developing molecular disease process hypotheses is given by linking network dynamics to the non-ideal patient, where diseases co-occur and interact. This information can also help with the development of targeted therapeutic alternatives and the repurposing of medications [16,93,97,108-116].

3.2. *Adverse outcome pathways*: The sequence of molecular and cellular events required to result in a harmful effect when a medication is administered to an organism is described by a model called an adverse outcome pathway (AOP). An AOP can be used to include information on biological interactions and toxicity mechanisms into models that explain how exposure to drugs may cause illness or injury. recommendations for route element assays based on cells or biochemistry that could be used to develop unique toxicity assessment procedures. Determine which steps in a toxicity process require more investigation. The AOP framework is a systematic approach to describing causal or mechanistic relationships between a series of intermediate crucial events, a molecular initiating event, and an unwanted outcome using the mechanistic data currently available. The AOP framework provides recommendations for creating efficient and useful alternative testing techniques [36,66,67,117-120].

Building on current biology and toxicological understanding of the connection between two toxicity pathway anchors—the molecular initiating event (MIE) and an AO—the creation of AOPs is an international endeavor. The first interaction or interactions between a stressor and a biomolecule on or within an organism's body are represented by the MIE. As the last stage of an AOP, the AO is a key event (KE) that should be taken into account when regulatory decisions are made regarding chemical safety (i.e., corresponding to an apical endpoint or measurements, such as developmental neurotoxicity, carcinogenicity, reproductive toxicity, etc., that are done in a test guideline study). However, by capturing the interaction (e.g., covalent binding, hydrogen bonding, electrostatic contact, etc.) between a chemical and the biomolecules (e.g., DNA, proteins, etc.) within an organism, the MIE serves as a catalyst for the subsequent steps of the process. Macromolecular interactions, cellular reactions, organ reactions, and organism reactions are examples of KEs that are typically depicted in an ascending order of biological organization, starting at the cellular level and progressing to the tissue/organ level before finishing at the organism level [11,36,67,90].

4. Cardiovascular system and the potential diseases

Researchers can enhance the effectiveness of customised therapy and understand phenotype-genotype interactions by understanding how human genetic variations impact disease. Although some genetic markers have been linked to the risk of disease, many remain unidentified. In this work, we propose a pathway-based strategy to uncover new molecular pathways linking genetic variations and diseases and to broaden the associations between diseases and genetic variants (**Figure 2**). The circulatory system, often known as the cardiovascular system, is responsible for moving blood throughout the body. The heart, arteries, veins, and capillaries make up the circulatory system. Cardiovascular disease (CVD) is currently the leading cause of death in the US. However, there are some things we may do to reduce your chance of developing these conditions. If they do occur, there are many therapeutic alternatives available. The symptoms, treatment, and prevention of diseases linked to cardiovascular disease (CVD) are very similar. A wide range of disorders are referred to by the broad term CVD. Some of these may appear concurrently or spread to other members of the group [121–125].

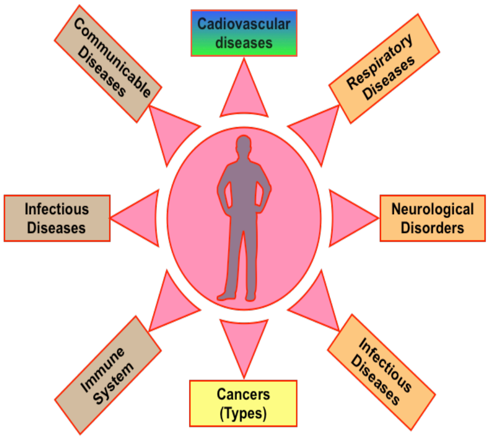

Figure 2. Potential diseases associated with human. There are huge number of human diseases but the diseases presented here are the major and the most common diseases.

Angina, which is chest pain brought on by a decrease in blood flow to the heart, arrhythmia, or an abnormal heartbeat or heart rhythm, and coronary artery disease, which affects the arteries that supply the heart muscle; congenital heart disease, in which there is a structural or functional issue with the heart from birth; A heart attack is an abrupt disruption in the heart's ability to receive oxygen and blood. A decrease in blood flow to the heart is a defining feature of heart failure[126-131]. The walls of the heart muscle swell in dilated cardiomyopathy, a form of heart failure that makes it challenging for the heart to relax, pump blood, and maintain electrical stability. During contractions, blood leaks back through the heart's mitral valve; a section of the valve bulges into the left atrium; hypertrophic cardiomyopathy, where the heart's muscle walls thicken and issues with relaxation, blood flow, and electrical instability arise; and hypertrophic cardiomyopathy (the condition that affects the blood vessel that transports deoxygenated blood to the lungs). Aortic stenosis, or narrowing of the aortic heart valve, impairs blood flow. Atrial fibrillation is a type of irregular heartbeat that raises the risk of stroke. Rheumatic heart disease, which results from strep throat, can impair heart valve function and create inflammation in the heart. Radiation heart disease arises when radiation exposure to the chest affects the heart valves and blood vessels [89,132-135].

Vascular diseases, which include several CVDs, affect the arteries, veins, and capillaries all over the body as well as those close to the heart. Peripheral artery disease (PAD) is a condition that affects the arteries, causing them to constrict and lowering blood flow to the limbs. A type of bulge or protrusion that has the potential to rupture and cause bleeding is called an artery aneurysm. On the inside walls of blood vessels, a form of plaque known as atherosclerosis forms, narrowing the channels and preventing oxygen-rich blood from flowing freely. Renal artery disease impairs blood flow to and from the kidneys, which leads to high blood pressure. Raynaud's disease causes arterioles to spasm, which momentarily lowers blood flow. Leg edoema and varicose veins are caused by peripheral venous disease, which is damage to the veins that return blood from the arms and feet to the heart. A blood clot that penetrates the brain can cause an ischaemic stroke, a type of cardiovascular illness. If a venous blood clot breaks free and hits the pulmonary artery, it could be fatal. One of the most common CVDs is blood clotting issues, which occur when blood clots develop too quickly or too slowly, causing excessive bleeding or clotting. Leg gangrene can be caused by inflammation and blood clots caused by Buerger's disease. While lifestyle changes might help manage many CVD health conditions, others can be lethal and necessitate emergency surgery [89,133,136-139].

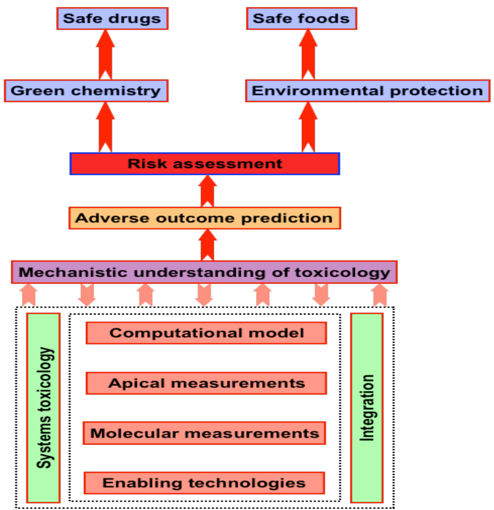

Figure 3. Toxicity, risk assessment, and integration for systems-level understanding.

5. Measurements

The way a medicine interacts with a cell, tissue, or organism determines how dangerous it is. We know that different people react differently to the same dosage of a medicine based on a number of factors, including body weight, age, and gender. Toxicology is therefore usually quantified at the population level. The likelihood of the population's result is then assigned to a single member of the population. Chemical structure and properties, metabolic transformation, internal dose, (ii) macromolecule binding, (iii) altered gene/protein expression, (iv) tissue function and homeostasis, (v) impaired development, disease, and lethality, and (vi) disease and death rates are among the most frequently used measurements. Quantification of physiological and histological defects (**Figure 3**), epidemiology, phenotypic data, computational biological network model, ligand binding and adduct formation, and more.

6. Enabled technologies and multi-omics

In toxicology, systems biology makes use of extremely potent high-throughput techniques and platforms, like "omics" technology. After almost 13 years and a significant financial investment, the first human genome sequence was finished in 2003. Genome sequencing can now be finished in less than a day thanks to advancements in technology. It has also enabled the management and analysis of massive volumes of data at various levels, including genes, transcriptomes, proteins, and metabolism. These technologies are currently being used by researchers from a variety of disciplines to offer a more thorough approach to toxicity and disease [32,145-147]. Because traditional toxicological endpoints can be insensitive and only provide a limited amount of information about toxicological mechanisms, it is necessary to combine apical data with high-resolution measurements using molecular profiling (omics) approaches. Robust computer analytical techniques are required to extract useful toxicological insights from these data. One example of a single-omics technology that can only identify one kind of biomolecule is transcriptomics, which can only identify alterations in a relatively little portion of the biological cascade. Therefore, single-omics analyses can help identify biomarkers for specific exposures, but they cannot provide a comprehensive understanding of toxicity or pathways leading to adverse consequences. The integration of many omics data sets offers a significant improvement in detecting this pathway reaction to a toxicant because of the increased amount of data and, more importantly, the greater understanding of the system. Specific biomolecule types, such RNAs, will be measured using a single-omics approach to transcriptomics. A single-omics approach frequently only finds a subset of biomolecules with comparable physicochemical characteristics rather than identifying the full kind of biomolecule (**Figure 4**)[13,103,148-150].

6.1. High Content Screening: In the mid-1990s, high-content screening was developed as a promising method to speed up drug discovery by looking at the intricate physiology of a cell or organism. High content screening (HCS) has gained popularity recently due to the unprecedented development of automatic microscopes with autofocusing, image acquisition and real-time analysis of cellular samples in multi-well microtiter plates, single-cell informatics techniques, and a biology toolbox filled with chemical probes, dyes, and antibodies. The role of HCS in a number of processes, including protein localisation, cancer cell susceptibilities, and complex organism phenotypes, has also been established. In connection with the HCS experimental designs, fluorescent probes, automation and miniaturisation, image data, image processing and segmentation, phenotypic trait quantification, image analysis, and picture collection were all investigated [14,90,151-155].

The HCS diagram The HCS assay design includes the creation or selection of cell models, incubation with test chemicals or genetic reagents, image capture, and image analysis. After the data has been analysed, the findings need to be interpreted. In drug discovery and development, HCS is useful for target identification, mechanism of action (MOA) research, secondary confirmation, first compound screening, and in vitro toxicology. HCS can be used to find genes required for certain biological processes based on a genetic perturbation screen using a genome-wide RNA interference (RNAi) screen. The use of HCS as an in vitro toxicological method to test drug toxicity and identify MOAs has resulted in a significant reduction in the use of animals in toxicological testing. Animal research is costly, low-throughput, and often fails to predict human toxicity [13,141,156,157].

6.2. Genomics: Genomic toxicology examines the relationship between genes and environmental stress and disease development by combining classical toxicology with transcript, protein, and metabolite profiling. Certain exposures or the consequences of certain diseases have revealed patterns of altered molecular expression that reveal the behaviours and disease-causing effects of several toxicants. Despite these advancements, scientists still face challenges in identifying the molecular basis of toxicity[4,12,22,158-160]. It is believed that the field of toxicology is evolving into systems toxicology, which will allow us to use toxicogenomic responses in one species to predict the modes of action of similar drugs in other species and explain all toxicological interactions that occur within a biological system under stress. The study of toxicogenomics, which looks at how an entire genome responds to toxins or other environmental stimuli, has grown as a result of these discoveries. The three primary goals of toxicogenomics are to clarify the molecular mechanisms of toxicity, discover useful biomarkers of disease and exposure to toxic substances, and comprehend the connection between environmental stress and human disease susceptibility [161–165].

6.3. Transcriptomics: cDNA microarray hybridisation and analysis for transcriptomics Gene-expression profiling techniques for toxicogenomics research were the first to use cDNA microarrays. Even while syntheticoligonucleotide microarrays, both short and long, are rapidly displacing cDNA technology, the basic concepts behind the two methods are essentially the same. Sequence-verified clones that represent the 3′ ends of genes are used to create cDNAs, which are then either synthesised in-situ or spotted onto glass slides using a robotic arrayer. Each RNA sample undergoes dye-conjugated dUTP (deoxyuridine triphosphate) following reverse transcription with an oligo-dT (deoxythymine) primer. The fluorescently tagged cDNAs are subsequently hybridised to the microarray and scanned using a fluorescence laser. The raw pixel intensity images from the scanner are analysed to identify targets on the array, measure the local background, and subtract it from the target intensity value [166-168].

6.4. Proteomics: Proteins are identified by mass spectrometry after global protein-stratification systems, like PAGE, are employed in a well-known proteomics approach. Two-dimensional PAGE separation by mass and charge can be used to nearly homogenise hundreds of proteins. This separation is necessary for both enzyme digestion and mass spectrometry identification, which both require different peptide-fingerprint masses or amino-acid sequence tags. When proteins are separated using liquid chromatography rather than PAGE, a new and promising platform that integrates multidimensional liquid chromatography can be used to fractionate and simplify the protein mixture before mass spectrometry or tandem mass spectrometry is used to sequence the peptides. This procedure, which isolates tens to hundreds of thousands of low-molecular-weight fragments that comprise a proteome, is being improved by Surface Enhanced Laser Desorption/Ionization (SELDI) time-of-flight mass spectrometry [148,169-172].

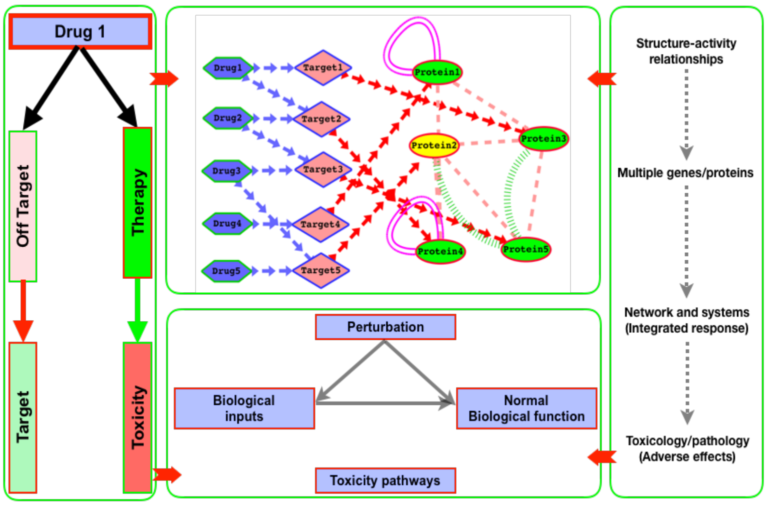

Figure 4. *Integrative toxicology and systems toxicology on several levels. Systems toxicology could play a role in drug development and discovery. Both in the early (discovery) and later (development) stages of the drug pipeline, systems toxicology has the potential to provide significant value. The ideas presented in this evaluation have several potential application areas.*

6.5. Metabolomics: To find metabolites in chemical pathways or classes, quantitative analytical methods have been developed. Many people support the practice of metabolite profiling, often known as metabolomics. The use of nuclear magnetic resonance (NMR)-based semi-quantitative metabolic fingerprinting to identify extremely prevalent metabolites is known as "metabonomics". While NMR peaks provide structural information about the metabolites, mass spectrometry peaks provide similar molecular weights. Additionally, new mass-spectrometry methods for breaking down the parent molecule can be created, which will allow metabolites to be identified by analysing fragmentation patterns [8,17,153,173-175].

6.6. Lipidomics: Lipidomics is a relatively young field of biological research that focusses on the study of complex lipidomes. A lipidome is a comprehensive and quantitative description of a group of lipid species present in a living organism. Thousands of cellular lipid molecular species pathways and networks are identified and measured at the systems level, along with their in vivo interactions with other lipids, proteins, and other moieties. Membrane-lipidomics is the quantitative and comprehensive study of the lipid components of membranes. The structural characterisation and measurement of low abundance bioactive lipid species are covered in mediator-lipidomics [150,176-178].

7. Multidisciplinary fields and Scientific/Multi-level integration

The sheer number of different methodologies available to the researcher is perhaps the most perplexing part of systems toxicology for those who are unfamiliar with it (**Figure 4**). As a result, you must first determine what you already know and what you intend to learn from a systems toxicology approach. You will be in a better position to decide which plan or strategies are ideal for you once you have addressed all of these concerns. Even though there are many distinct kinds of decision trees, the following questions are applicable to all of them. Does the mechanism need to be understood by me? (2) Do you know a lot about the biology you're studying? (3) Does the biology under review have a firm understanding of it? (4) Is it possible to investigate biology at the molecular, cellular, or organismal levels? If you answered "no" to the first question, a relationship strategy might be the best option. Relationships between network components are predicted using a relational approach, which does not require knowledge of the mechanical underpinnings [68,82,83,179-194].

These correlations can be used to predict how chemicals or proteins will interact, as well as to predict the toxicity of a molecule based on its structural components. To find associations, you can utilise statistical correlations, guidelines, or a collection of literary data. If the answer to the second question is also no, relational techniques might be your best option because you can't mechanically articulate what you don't know. If you answered "yes" to the second question, then a modelling technique would be acceptable. If not, the third and fourth questions will help you determine what kind of model to use. A model's classification as quantitative (based on known network connectivity and kinetic/abundance values) or qualitative (based on known network connectivity but no kinetic/abundance values) depends on the response to the third question [82,112,113,195-198]. Quantitative models can accurately replicate biology by predicting dose- and time-courses with physiologically meaningful values. However, qualitative models do not use real data to predict how a system would behave. Both approaches are valid, and the availability of the data often influences the decision between them. How much reductionism is required in the model will also depend on the answer to the fourth question. As a model becomes more complicated and tries to reproduce more biology, it becomes more demanding in terms of the biological data needed to build the model and the computational power needed to execute it. Larger models sometimes compress complex biological sub-systems into simpler, easier-to-model pieces, therefore there needs to be a balance struck between model complexity and size [103,113,199].

8. New Paradigm for risk assessment

Based on the nature and seriousness of the danger, risk assessments can vary from simple hazard classification (e.g., is it genotoxic or not) to quantitative risk assessments that determine the type and urgency of any risk-management initiative. Even while modern methods have been beneficial to society, there is growing genuine and perceived fear that the assumptions made in risk assessments may be gravely incorrect. The nature of the dose-response relationship under human-relevant exposures, the existence of biological thresholds and at what end points, the role of factors like life stage on response, and population variability in toxicological response are only a few of these [13,57,200,201]. Despite extensive research, some of which involved vast numbers of animals, these problems remain unresolved. This flaw arises from the fact that all experimental observations are constrained by the study's power. For these problems to be fully resolved, a mechanistic approach will be required. As was previously said, Systems Toxicology has a lot of potential to help solve these problems by offering quantitative mechanistic models and data. Systems Toxicology can provide a comprehensive mechanistic understanding of the toxicological consequences to predict chemical responses. A well-written description of a system should be able to predict behaviour even in the absence of experimental data; that is, the system will show emergent properties, which are new patterns and characteristics that come from the system's intrinsic structure.

Making such models is a challenging and complex task. They will therefore be unavailable for use in risk assessment for some time. Furthermore, because risk assessors are usually unfamiliar with the relevant mathematical procedures, they automatically reject the use of computationally costly methods. This will necessitate a phased shift. Modifying the more widely used mode of action/adverse effect pathway model is one way to achieve this. A series of measurable critical events known as a MOA/AOP, which are usually insufficient on their own, result in a toxicological reaction to a chemical. It should be able to construct systems toxicology in a modular manner by beginning with a systems-based model of a single important event and integrating it with dose-response data for the other important events at the operational level. This approach made it possible to characterise each important event until the reaction was completely characterised by progressively adding systems-based modules. The primary benefit of this approach is the scientific validation of every stage. This will also enable systems toxicological integration into risk assessment by helping risk assessors to gradually grow comfortable and familiar with such data.

Many non-animal test methods are being developed, such as the previously discussed high-throughput ToxCast program [13,59,202]. However, these platforms alone are unlikely to achieve risk assessment objectives beyond hazard identification. To go further, more quantitative techniques would be required, and Systems Toxicology would be ideal for this. However, developing sufficient models will be a challenging and time-consuming undertaking. By integrating data from in vitro and other methodologies into a systems-based description of significant events, the mode of action can act as a translational link, allowing for the gradual development of a comprehensive Systems Toxicology characterisation of the organ and, ultimately, the organism [13,59,202,203].

9. Future perspectives and conclusions

There is about to be a paradigm change in the way toxicological evaluations are conducted. The use of contemporary molecular analytic technologies to elucidate toxicity pathways is a crucial component of the new toxicology, which is made possible by a number of variables. First, molecular measurement methods have become more accessible and capable of evaluating the functioning of biological networks inside animals, organs, tissues, and cells. In contrast to the chemical-by-chemical approach of the previous forty years, which is expensive, time-consuming, and requires the use of thousands of animals, the second is the increasing affordability of high-throughput and high-content characterisation techniques that can be used to rapidly characterise thousands of chemicals. The scientific community's increasing availability to computational power, data storage, and information management technologies is the third enabler, which makes using complex Systems Biology models easier. The fourth enabler is the development of appropriate in vitro test methods to complement and eventually replace animal models.

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