TOXICITY ASSESSMENT AND EPIDEMIOLOGICAL STUDIES OF CHEMICAL SUBSTANCES – AN OVERVIEW

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Abstract:

Humans are exposed to many chemicals on daily basis but mostly toxicity obtained from single chemical exposure. Many factors are to be considered such as duration, route, frequency, timing of exposure, concentration while determining toxicity. To better understand the toxicity of chemical mixtures, a proper model system is essential. Shortcoming model systems are facing difficulties to find out appropriate balance of rigor and reproducibility in mixture toxicity studies. Certain questions will arise while comparing single to mixture toxicity. The most sensitive cases where toxicity leads to occur at lower doses is called as critical effect. They are based on the synergism, additivity, antagonism and potentiation. For toxicity evaluation to be more accurate, dose or concentration relevance should be considered and be below the threshold. Previously the work on chemical substances in done by the technology and methodology of the time but the recent studies are based on the progress of chemical substances in the mixture toxicology studies. Epidemiological risk assessment test are based on the chemical analytical tests. The evaluation of chemical substance is done by the quantitative or qualitative results. Amount of dose that is given to particular individual should be known for the study during exposure. This review consists of methods to determine the toxicity of using certain chemical substances and their epidemiological studies and functioning of chemical substances on human organs.

Keywords: Toxicity, Chemicals, Dose, Synergism, Antagonism.

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INTRODUCTION:
The branch of toxicology deals with poisons, their effects, and remedies. The crucial process for creating novel medicines and enhancing the therapeutic potential of already existing compounds is toxicology screening. The USFDA (Food and Drug Administration) is essential for the development of new drugs for pharmacological activity and toxicity potential in animals. In recent times humans are exposed to many chemicals through multiple routes. Chemical mixtures from simple combinations to multiple combinations are known as cumulative exposure.

Newly developed approaches not considering real-life exposure scenarios for risk assessment. So, particular attention is to be taken at certain life periods for toxicity assessment from chemical exposures. Toxicity studies are mainly used to examine the adverse effects of chemical substances on humans.
Some chemicals like inorganic fibers obtained from mining and processing ores which contain major fractions of naturally obtaining fibrous minerals like asbestos and zeolite. The benefit of the entire mixture technique is that it can evaluate the potential for interactions and toxicity of unknown mixes with unknown compositions.

This whole-mixtures approach is used for many kinds of situations of unknown composition but is available for a limited number of mixtures. It measures the combined effect of complete chemical substances in the human body. This method is applied when the composition of the situation is unclear or varies, such as when chemical substances are present on the surface of the water.

The critical effect is the state that occurs at lose dosage situations and it has no prolonged adverse effects. In Pharmaceutical safety assessment these conditions that are having low adverse effects are called as No Observed Adverse Effect Levels and these are used to identify the safety of the chemical substances [1].

Paracelus, father of toxicology also a physician, alchemist, astrologer determined specific chemicals that cause toxicity for plants and animals. Mathieus Orfila, a Spanish physician determined relationship between toxins and their biological characteristics and also identified organ damage caused by toxins.

J.W.Trevan startled the use of animals to estimate lethal dose of individual chemicals. In order to test for eye and skin irritation using rabbits, John Draize created a procedure after obtaining the LD50 for each particular drug.

The technique is frequently employed to examine the effects of chemicals on the skin and eyes. By giving chemicals to rats and mice daily doses, the USNCI (US National Cancer Institute) has devised a test to discover compounds that cause cancer. In humans, thousands of babies are born with birth defects due to the use of Thalidomide drug.

Following this, the entire regulatory organization focused on defining the toxicity profiles of all pharmacological drugs used for routine patient care. The certain guidelines need to be followed in toxicity testing under Organisation for Economic Co-operation and Development (OECD) [2].

DEVELOPMENTAL TOXICOLOGY
In this field, there are many categories to identify and evaluate the testing procedures to understand the mechanism of toxicity. Present testing procedures have only limited availability of the exposure of the chemical and its period of effect and exposure in children.

It exemplifies how widely integrated predictive models employ readily available toxicologically related materials. explains the requirements that must be completed for meaningful science to be produced. The dependability and reproducibility of modern science have been heavily contested. Biology has a high risk of false positives and false discoveries due to its high level of variability [3].

The NESARC and NCS-R surveys' 12-month and lifetime prevalence rates of alcohol and drug misuse and dependence. The lifetime rates are, as anticipated, significantly higher than the 12-month rates in both investigations.

The prevalence rates of alcohol use disorders in the NESARC study (4.7 % and 3.8 % for 12 months, and 17.8 % and 12.5 % for a lifetime, respectively) were significantly higher than those estimated in the NCS-R survey (3.1 % and 1.3 % for 12 months, and 13.2 % and 5.4 % for a lifetime, respectively), most likely due to differences in the criteria used to assess alcohol use disorders. However, there were significant similarities between the two studies' estimations of drug use disorders.

These results highlight the high prevalence of substance use disorders among U.S. people over the course of their lifetimes, which is comparable to the

prevalence rates of other significant mental illnesses like depression and anxiety.

Table 1. Estimation of Alcohol Abuse and Drug Abuse

Substance	12 months	Lifetime	12 months	Lifetime
Alcohol abuse	4.7 (0.2)	17.8 (0.5)	3.1(0.3)	13.2 (0.6)
Dependence	3.8 (0.1)	12.5 (0.4)	1.3 (0.2)	5.4 (0.3)
Drug Abuse	1.4 (0.1)	7.7 (0.2)	1.4 (0.1)	7.9 (0.4)
Dependence	0.6 (0.1)	2.6 (0.1)	0.4 (0.1)	3.0 (0.2)

Genes underlying complex illnesses are now being identified, and the discipline of genetic epidemiology is using its tools more frequently to determine how much genetic and environmental variables contribute. Three following particular factors set genetic epidemiology apart from its parent fields of human genetics and epidemiology:

- Population-based research is its main priority.
- Identifying the combined impact of genes and environment is its objective.
- The inclusion of a disease's underlying biology in conceptual models.

  Five of the major applications of genetic epidemiology in order to advance our understanding of substance use disorders are
- The creation of population-based databases for substance use disorders, which will become more and more important for assessing the plethora of genetic tests that will result from developments in human genetics and the Human Genome Project.
- Finding more uniform subcategories of complicated illnesses through study on high-risk families.
- To determine strength, family patterns of affected and unaffected probands are examined.
- Risk classification at the individual and population levels.
- By combining the methods of genetic epidemiology, behavioral neuroscience, developmental psychology, and neuroscience, a broader conception of environmental elements that may be significant mediators of expression of genetic risk for substance use disorders can be developed [4].

METHODS TO DETERMINE TOXICITY
Acute toxicity testing
Animals are habituated to laboratory conditions and are supplied with sufficient oxygen. The animal is subjected to the test drug for at least 4 hours, and is then monitored for 14 days. Food and water are not provided during the exposure period. During this period, the animal is observed for, convulsions, coma, salivation, tremors and sleep. Mortality is observed. After that scarify the animal for histological and pathological changes and are evaluated. Ophthalmic and Dermal preparations are done by using Draize test. The techniques are used to assess if chemicals and pharmaceuticals are dangerous to rabbits. In eye irritancy test, test substance of 0.5ml is administrated into animal eye, the animal is restrained for 4hrs. Redness, Swelling, Ulceration, Blindness are treated and monitored for 14 days.

In the skin irritancy test, an animal's skin is exposed to 0.5 mg of the test drug, and the erythema and edema are tracked for 14 days. The animals are sacrificed at the conclusion of the investigation for pathological conditions.

Skin sensitization test
This test is observed in guniea pig. This can be obtained by Draize test. LLNA (local lymph node assay) method is alternative to Draize test and is widely accepted test it has all regulatory requirements. In this experiment, a mouse's ear was exposed to test compounds for three days in a row, and the proliferation of lymphocytes in the draining lymph node was assessed.

Repeated dose toxicity testing
A rodent of any gender of age 5-6 weeks is taken. The test substances administered orally for a period of 28 days. If this route is not possible then parenteral route is preferrable. Test substance should be administered regularly at specific time. There should be a 20% weight difference between the animals. The study plan should include a satellite group, and this group must have both a control and a high dose group. It is important to record baseline variables such as behavioural and physiological data. This study's assessment of human safety information is crucial, and at its conclusion, tissues from the majority of the organs are removed, and histological changes are noted [5].

Carcinogenic testing
Both rodents and non-rodents are involved in this test. The growth of tumours and signs of toxicity are monitored in the experimental animals both during and after exposure to the test drug. If they are not discovered, the test may be stopped after 18 months for mice and 24 months for rats. After a year, a haematological investigation is done and the animals are killed if they are healthy. The animals are sacrificed for pathological conditions.

Mutagenicity testing
This test is used to determine the microscopic changes in the base sequence of DNA, chromosomal aberrations, duplication and insertions. Particular type of mutations leads to carcinogenesis. The development of mutagenicity is important for new drug development process. Test mostly includes bacterial reverse mutation assay. Two or three distinct bacteria are used in this in vitro setting to cover the end points of gene alterations. For the purpose of determining the risk assessment for test compounds on a case-by-case basis, in-vivo mutagenicity is dosage dependent.

EPIDEMIOLOGICAL STUDIES
Epidemiological studies are designed to predict whether the particular disease is result from exposure to a risk or toxicity factor. These are additionally vulnerable to various types of prejudice. When using chemical mixtures, it's important to understand how much each person has been exposed to the mixture's various components.

Exposure events are assessed for frequency, duration, and magnitude of exposure for mixture risk assessment it is important to identify whether multiple chemical mixtures are associated with disease phenotype in human population [6]. It is crucial to understand how each individual was exposed to each component when using chemical mixes in modern epidemiological investigations that use new and unique technologies. New methodological development will increase the scope and quality of epidemiological data.

Methodologies for preparing epidemiological evidence include systemic reviews and meta-analysis which will determine quality. Meta analysis will increase statistical power improve precision of effect size estimation. The process of identifying the kind and potential consequences for human health of exposure to chemicals in a contaminated environment is known as human health risk assessment.

New methodological developments will increase the scope and quality of epidemiological data on chemical substances which has the use of extracted data from multiple existing studies and also for disease markers. It is mainly used to determine the disease in the human population. Epidemiological studies are related to the type and kind of illness in people. Epidemiological evaluators investigate the relationship between the agent, host, and environment. These will identify the kind of disease that occurred among the human population.

These data can be applied to identify the bias that may occur in the threatened population. Testing the sample substances is the major contribution to epidemiological studies. Environment-Wide association Studies will estimate simultaneously the relationship between health outcomes and the range of chemicals. Exposomes are identified from personal information and environmental information.

The 21 case studies covering various component classes and environmental classes were found in a recent analysis of multiple case studies on the evaluation of the risks that chemical mixtures pose to humans and the environment. It also provided clear evidence that chemicals are to be studied further to determine risk not only in single chemical substance but also in mixtures. Human risk assessment is the process to estimate the nature of adverse effects in humans who are exposed to multiple chemicals.

In hazard identification scientists determined the types of health problems that a chemical can cause. In exposure assessment scientist attempt to determine the period of exposure of chemical individuals are exposed and the amount of chemicals and the way of exposure whether it is continuous or intermittent and how people are exposed by eating, drinking water and other liquids, breathing or skin contact. The last in risk determining gives the information developed in previous steps that will determine the risk of health effects in chemical exposure population. In this step scientists analyse the data developed during the exposure and the dose-response assessment which results in health risk which are expected to occur in chemical exposed population.

Dose-response relationship applied for studies where exposure is a concentration of agent and resulting information which is also known as concentration response relationship. The relationship is based on observed data from human clinical trials, epidemiological studies and animal studies.

By exposure assessment method determination of amount, duration and the pattern of exposure for many chemicals which are provided in the form of dosage. Risk characterization will help to identify that is which is cause by overdosage and the chemical risk which leads to cancer or other illness in general population [7].

MECHANISM OF HEAVY METALS IN HUMANS

Many significant efforts are going ahead to move beyond the traditional assessment of chemical toxic end points in laboratory animals for the evaluation of toxicity based on their mechanism and pathways along with quantitative determination of relevant parameters through invitro studies. Present *in-vitro* systems are suitable for studying and identification of different chemicals that are associated with various physiological process where hazard assessment is detected by testing cell lines.

Heavy metals are transported in the body through blood to tissues. Lead is delivered by red blood cells to the liver and kidney and also to teeth, bone, hair as phosphate salt. In kidney and liver tissue, metallothionein and cadmium attach to blood cells,

albumin, and other molecules. Manganese vapour travels from the blood to the lungs and then diffuses across the lung membrane to the central nervous system. In the colon, lipid-soluble organic manganese salts are dispersed for fecal excretion. For renal elimination, water-soluble inorganic salts are dispersed throughout the plasma and kidney. Blood carries arsenic, which builds up in the kidney, lungs, heart, and lungs. Receptors or certain pathways may get activated in response to one chemical or a group of chemical substances, starting particular intracellular processes like lesion healing.

In-vitro assessment of chemical compounds is difficult and it might be challenged through top-down or bottom-up methods.

As with arsenite-induced protein aggregation, heavy metals can make proteins clump together. The aggregate contains a wide range of proteins with high levels of metabolic, protein folding, and protein synthesis-related capabilities. The effectiveness of cellular absorption of these drugs' various biological action modalities determines how effective they are in causing protein aggregation in vivo.

Table 2. REGULATORY LIMIT OF HEAVY METALS

Heavy Metals	EPA limits in drinking water (ppm)	OSHA limit in workplace air (mg)	FDA limit in bottled water/food (ppm)
Arsenic	0.01	10	-
Barium	2.0	0.5	-
Cadmium	0.005	5	0.005
Chromium	0.1	1	1
Lead	0,015	0.15	-
Mercury	0.002	0.1	1
Selenium	0.05	0.2	-
Silver	0.0001	0.01	-
Zinc	5	5	-

Health Effects:
When humans are exposed to toxic chemicals divided into categories
1. Short-term effects
2. Long-term effects

Short-term impacts can be local or systemic and manifest quickly after exposure to a material at a relatively high concentration. Local effects happen where the toxicant and body make contact. Systemic effects are those that take place when a toxin enters the body from its first point of contact and negatively affects organs that are susceptible to it [8].

Long periods of time between exposure and harm are considered to have long-term impacts. Acute chemical

impacts on the body depend on the affected organ and chemical. Targeted organs are those where a chemical's major effects are expressed throughout a number of organs.

The organs of the body that are affected due to chemical exposures are:

Respiratory Tract:
The respiratory tract is the organ system with functional elements in direct contact with environment. The respiratory tract is divided into
1. Nasopharyngeal
2. Tracheobronchial
3. Pulmonary acinus

Nasopharyngeal:
It reaches the larynx from the nose. Mucous glands and ciliated epithelium line the interior of these tubes. The substantial inhaling particles are separated. and raise the air that is breathed in's relative humidity.

Tracheobronchial:
It functions as a conducting airway between the nasopharyngeal area and alveoli and is made up of the trachea, bronchi, and bronchioles. The ciliated epithelium that lines these passageways acts as an escalator to transport particles from the deepest region of the lungs to the mouth cavity where they can be eaten. In this step scientists analyse the data developed during the exposure and the dose-response assessment which results in health risk which are expected to occur in chemical exposed population [12].

Pulmonary acinus:
It is the principal site for gas exchange and the basic functional exchange of the ling. It is made up of tiny bronchioles that join to alveoli. Depending on their width, inhaled particles settle in the respiratory tract.

- Particles between 5 and 30 microns in size are deposited in the nasopharynx.
- Particles between 1 and 5 microns in size are deposited in the tracheobronchial area.
- Particles smaller than 1 micron are deposited in the alveolar area.

Certain inorganic particles are settled at the smaller regions of the lung.

Skin:
The largest organ on the body is the skin. It serves as a wall between the outside world and other organs. Sweat ducts and glands are found in the dermis layer of the skin. Chemicals can quickly pass via sebaceous and sweat glands. Although follicles and glands may permit the small amounts of chemicals and they mostly penetrate through epidermis which has a large surface area.

Central Nervous System:
Neurons have high metabolite rate. Inadequate oxygen flow to brain kills cells within minutes.

Kidneys:
The kidneys receive 20-25% of blood flow and have oxygen and nutrient requirements because of their workload.

CONCLUSION:
Toxicity assessment includes dose-response information for adverse effects. Toxicological technologies help to evaluate the chemical substances on the living organisms. These technologies are applies to improve the analysis of chemical hazards. Many chemicals are present in environment which are to be evaluated for potential toxicity. Priority must be given to choosing which chemicals to test for potential effects on public health. It is challenging to determine which chemicals to test because there are so many potential health consequences to look for and so many possible tests and test combinations.

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