Chapter

Cytotoxicity as a Fundamental Response to Xenobiotics

Grethel León-Mejía, Alvaro Miranda Guevara, Ornella Fiorillo Moreno and Carolina Uribe Cruz

Abstract

Cytotoxicity refers to the ability of a molecule or a compound to cause some type of cellular damage, of which some of the adverse effects that can occur include injuries to some structures or the fundamental processes involved in cell maintenance, such as survival, cell division, cell biochemistry, and the normal cell physiology. The potential for cytotoxicity is one of the first tests that must be performed to determine the effects of drugs, biomolecules, nanomaterials, medical devices, pesticides, heavy metals, and solvents, among others. This potential may be oriented in the mechanism under which it generates cell death, the dose, and the target cells that generate the response. The evaluation of the toxicologic and cytotoxic properties of the chemical substances through *in vitro* tests has become a competitive alternative to *in vivo* experimentation as a consequence of ethical considerations. Presently, there are numerous tests conducted to evaluate the cytotoxicity of a certain agent, the selection of which depends on the purpose of the study. In this sense, the present review provides a general overview of the different responses of a cell to xenobiotic agents and the different test that can be useful for evaluation of these responses.

Keywords: cytotoxicity, xenobiotic, apoptosis, biomarkers, cellular damage

1. Introduction

In the modern world, increasing industrialization continues to pose serious pollution problems [1]. Every year, several countries generate millions of tons of pollutants, which keeps adding to this interrelation among population, technology, resource consumption, and the environment—a situation which is becoming increasingly complex [2]. Although it is true that the reality is alarming, risk quantification and estimation strategies, based on studies of the effects of xenobiotics, are necessary not only for the conservation of the environment but also to acquire the knowledge about the factors that intervene in each specific case to enable foreseeing potential injuries [1]. Humans are exposed to a wide variety of foreign chemical substances, which we collectively call "xenobiotics" that includes natural compounds present in plant foods, such as synthetic compounds in medicines, food additives, and environmental pollutants. At present, the study on the effects of xenobiotics and the elucidation of their mechanisms of action on macromolecules has become extremely important [3, 4].

Cytotoxicity is defined as an alteration of the basic cellular functions that leads to detectable damage [5]. In this sense, the cytotoxicity that a xenobiotic induces may be a relevant point in the elucidation of the mechanism of action of the xenobiotic [6]. The analysis of cytotoxic effects is a fundamental strategy involved in the analysis of the xenobiotic–cell interaction in basic research, industrial development, and in the evaluation of therapeutic and toxic effects of chemical and biological products [5].

2. Cytotoxicity and genotoxicity

Genotoxicity is defined as the capacity of an agent, be it physical, chemical, or biological, to cause damage to the genetic materials or to alter the cellular components that influences the functionality and behavior of the chromosomes within the cell, leading to adverse biological outcomes [7]. Hence, the final cause of cell death may be related to DNA damage [8]. Specific cellular responses include cell cycle arrest and attempts to repair DNA [9]. The use of a specific repair enzyme complex depends on the type of DNA strand break or the chemistry of the adduct formed as well as the repair capacity of the affected cells [9]. If there is excessive DNA damage, an alternative is the action of p53 that activate apoptosis [8].

Apoptosis

One of the most obvious end points of action for several drugs and toxic xenobiotics is cell death [10]. Cell death is divided into two types: i) necrosis, which is characterized by the occurrence under the mechanisms of irreversible cell injury and is considered accidental and ii) apoptosis, which corresponds to programmed cell death that runs under control and is related to homeostasis of tissue growth [11]. The term programmed cell death was introduced for the first time in 1920, and, in 1972, the term apoptosis was coined by Wyllie and Currie, since then the mechanisms and molecular aspects by which this process is conducted have been described [12].

The extrinsic signaling pathway that initiates apoptosis has been so named since it involves interactions mediated by transmembrane receptors, and it has been described that this pathway is initiated by the binding of i) tumor necrosis factor (TNF) ligand to the receptor TNF, ii) TNF-related apoptosis inducing ligand (TRAIL) to death receptor-4 (DR4), and DR5 receptors, or 3) the fatty acid synthetase ligand to the FasR receptor. These associations are known to recruit adapter molecules such as Fas-associated death domain (FADD) or TNF receptor-associated death domain (TRADD), which activates initiator caspases-8 and -10 and, finally, the activation of executor caspases-3, −6, and − 7 that culminates in an apoptotic cell phenotype with characteristic physiological and morphological characteristics [11, 13].

In the intrinsic signaling cascade, a series of intracellular stimuli has been reported specifically in the mitochondria, which induces structural changes in the mitochondrial membrane, mainly due to the opening of the transition pores and alterations in the transmembrane potential entailing a release toward the cytosol of pro-apoptotic substances, which remains within the intermembranal space in a normal state [14]. These released components have been classified into two main groups consisting i) cytochrome c, Smac/diablo (second mitochondrial activator of caspases/direct inhibitors of apoptotic proteins (IAP) binding protein with low Propidium iodide (PI)) and the serine protease HtrA2/Omi (high-temperature requirement): these releases lead the apoptotic cascade via caspase activation. It has been discovered that the release of cytochrome c activates the

apoptotic protease activating factor-1 (Apaf-1 protein) and procaspase-9 in addition to ATP, thereby establishing a protein complex called as the apoptosome, which in turn activates caspase-3, initiating the effector pathway of apoptosis. On the other hand, the Smac/Diablo and HtrA2/Om proteins promote apoptosis by inhibiting IAP such as cIAP1, cIAP2, and XIAP. ii) Apoptosis inducing factors, endonuclease G, and caspase-activated DNase: the latter are released into the cytosol, enter the nucleus, and fragment the DNA. The importance of DNA degradation by Ca^{2+} and Mg^{2+} dependent endonucleases is that they generate fragments of 180–200 base pairs; this pattern of fragments is highly specific and an extremely clear factor that differentiates this type of programmed cell death with necrosis, which does not present a degradation pattern or specificity in the fragment sizes [11, 13, 14].

Autophagy

Autophagy is an intracellular degradation process that is characterized by the formation of double membrane vesicles called as autophagosomes; these vesicles sequester the cytoplasmic material and later fuse with the lysosome (that contains hydrolytic enzymes), forming the autophagolysosome or autolysosome, where the degradation of the invaginated material occurs [15]. The amino acids and small molecules that are generated through autophagy are returned to the cytoplasm for the generation of energy and for the synthesis of new proteins and biomolecules [16]. The main inducer of autophagy is nutrient deficiency; however, it has been reported that the activation of autophagy is a cell survival mechanism against various stress conditions, including oxidative stress, inflammation, protein aggregation, endoplasmic reticulum stress, metabolic stress, the presence of pathogens, and changes in the mitochondrial function [17]. Autophagy plays an important role in cell and tissue homeostasis by contributing to the generation of energy from degradation events, which plays a role in the quality control of proteins and organelles, in the elimination of long-lived proteins and pathogens, as well as in the regulation of cell death [15, 16].

The central machinery of the autophagy process is composed of >30 proteins, including the so-called Atg. The autophagy pathway proceeds through five phases: (i) nucleation, which is the formation of a double membrane structure or an isolating membrane called the "phagophore"; (ii) the expansion of the phagophore membrane by the incorporation of the LC3-II protein; (iii) the maturation of this structure in the autophagosome and the sequestration of cytoplasmic material to be degraded; (iv) the fusion of the autophagosome with the lysosomes, which results in the formation of autophagosome and autolysosomes and, finally; (v) the degradation of biological materials sequestered by the hydrolytic enzymes of the lysosome and the recycling of the molecules (above all amino acids, lipids, sugars, and nucleotides) [17, 18].

The nucleation and formation of the phagophore is initiated by the serine and threonine kinase unc-51-like autophagy activating kinase 1 (ULK1). Once the ULK1 complex is activated, it activates the phosphatidylinositol 3-kinase (PtdIns3K) complex that includes members such as Beclin 1, Atg14, Vps15, Vps34, and Ambra1. Two ubiquitin-like conjugation systems participate in the expansion of the phagophore membrane, until the closure of the double membrane vesicle to form the mature autophagosome, which are Atg12-Atg5-Atg16 and Atg4-Atg7-Atg3/LC3-PE (phosphatidylethanolamine) [17, 18]. Eventually, the autophagosome fuses with the lysosome, and the sequestered material is degraded by lysosomal enzymes (such as cathepsins, glucosidases, lipases, and sulphatases). The components of degraded biomolecules, for example amino acids, are returned to the cytoplasm to derive energy and for the synthesis of new biomolecules [17, 18].

3. Cytotoxicity test

Within the battery of *in vitro* tests that are useful and necessary, alternative toxicology methods for the registration or application of clinical trials of a given substance are referred to as cytotoxicity tests; these tests are capable of detecting, through different known cellular mechanisms, the adverse effects of interference with structure and/or properties essential for cell survival, proliferation, and/or functions [19, 20]. These tests include the integrity of the membrane and the cytoskeleton, metabolism, synthesis, degradation, release of cellular constituents or products, ionic regulation, and cell division [19, 20].

The sensitivity and speed of the damage analysis at the cellular level increases its practical value when simple cytotoxicity markers are used, such as in the determination of viability by exclusion of fluorescent and non-fluorescent dyes. There is a wide availability of markers for intracellular and extracellular structures and functions, as well as to examine several of these markers simultaneously in the same cell to allow analysis at individual cell level [19, 20].

Among the best-known tests are neutral red uptake assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) reduction, clonogenic assay, sulforhodamine B, lactate dehydrogenase (LDH) release colorimetric assay, annexin V/propidium iodide (PI) staining, TUNEL, and kenacid blue and resazurin binding assay. **Table 1** shows *in vitro* key studies on cytotoxicity induced by xenobiotics using different assay.

Neutral red uptake assay

This test is a measure of the toxicity of a compound in the short or long term, which is determined by the release of a dye (i.e., neutral red) due to the loss of cell viability. In this sense, the fact that a compound is cytotoxic regardless of its action mechanism must be considered, if this interferes in the process of cell division and multiplication [34, 35]. This interference leads to the reduction in the speed of cell growth, which is reflected in the number of cells present in the culture. The degree of growth inhibition related to the concentration of the compound being evaluated is an index of toxicity [34, 35].

Neutral red is taken up by cells (specifically by lysosomes and endosomes) and, as the cell loses viability due to the action of the compound being evaluated, the dye is released into the medium, since only viable cells can retain the dye inside. The amount of neutral red dye that remains after exposure within the cell is then determined and the concentration that produces 50% inhibition of cell growth is then calculated [34, 35].

The MTT reduction assay

This method is simple and is used for the determination of cell viability, given by the number of cells present in the culture, which can be measured based on the formation of a colored compound as a result of a reaction occurring in the mitochondria of the viable cells [35, 36].

MTT is a compound belonging to the family of tetrazolium salts that is soluble in water and a yellow color. The metabolic activity of cells includes mitochondrial succinic dehydrogenase enzyme, but cytosolic reductases or reductases from other subcellular compartments may also be involved. The resulting reduced coenzymes (NADH and NADPH) will convert MTT to its insoluble formazan form [37]. When reduced, MTT becomes a compound purple and insoluble in water. To quantify MTT, it is usually dissolved in an organic solvent such as dimethyl sulfoxide (DMSO). The amount of reduced MTT is quantified by a colorimetric method, since the color changes from yellow to purple as a result of the reaction [35, 37, 38].

Reference	Xenobiotic	Cells used	Assay used	Outcome(s)
[21]	Pesticides Deltamethrin Fenitrothion, Fipronil, Lambda-cyalothrine, and Teflubenzuron	Caco-2 cells	MTT cell viability assay	Cytotoxic effect of Deltamethrin, Fenitrothion, Fipronil, Lambda-cyalothrine, and Teflubenzuron alone or in combination in human intestinal Caco-2 cells.
[22]	Malathion	N2a mouse neuroblastoma cells	MTT cell viability and LDH release assay	The non-cholinergic effect of malathion may be mediated by apoptotic cell death via autophagy and lysosomal membrane permeabilization induction in N2a cells.
[23]	X-ray	Human hepatocellular carcinoma cells	MTT and clonogenic assays	Cell autophagy was significantly increased after ionizing radiation combined with hyperthermia treatment. Autophagic cell death may be due to the increased intracellular ROS.
[24]	Gamma radiation	Human breast cancer cell line (MCF-7)	Clonogenic cell survival assay, cell viability using trypan blue staining and apoptotic cell death using the TUNEL assay	The dose and time dependence inducing a significant apoptotic death.
[25]	Heavy metals	Human sperm cells	WST-1 and XTT	Harmful effect of $CuSO_4$ and $CdCl_2$ on human spermatozoa.
[26]	Heavy metals	HT-22 cell line	MTT assay and Annexin V-FITC/ Propidium iodide (PI)	Metal mixtures showed higher cytotoxicity compared to individual metals.
[27]	Chemotherapeutic drug Cyclophosphamide	Monocyte Macrophage Cell Line Raw 264.7	MTT assay	A reduction in cell viability was found in Raw 264.7 cell line indicating the cell cytotoxicity.
[28]	Chemotherapeutic drugs Paclitaxel Docetaxel Oxaliplatin Bicalutamide Anastrozole	HT-29 and HeLa cells	MTT Assay	Dose-response cytotoxicity findings. Favorability of *in vitro* assay for the selection of chemotherapeutic drugs for greater clinical effectiveness.
[29]	Aminated polystyrene, zinc oxide, and silver nanoparticles	HeLa cells	MTT, Alamar blue, and neutral red assay	All nanoparticles tested resulted in the decrease in cell viability, increased intracellular ROS production and induction of cell death by caspase-mediated apoptosis.

Reference	Xenobiotic	Cells used	Assay used	Outcome(s)
[30]	Copper oxide, copper-iron oxide, and carbon nanoparticles	Human hepatoma HepG2 cells	MTT and neutral red assays	There was increased cytotoxicity, mutagenicity, and mitochondrial impairment in the cells treated with higher concentrations of the nanomaterials, especially the copper oxide nanoparticles.
[31]	Dental universal adhesives	Monocyte/ macrophage peripheral blood cell line	XTT assay	Some of the tested adhesives showed significant cytotoxic and genotoxic effects.
[32]	Perfluorocarbons for intraocular use	BALB/3T3, ARPE-19 cell lines	MTT, neutral red uptake, and TUNEL assay	Qualitative evaluation showed that cytotoxic control induced apoptosis, severe reactivity zones, and cytotoxicity according to ISO 10993-5 in all tested conditions.
[33]	Polysiloxane-based polyurethane/ lignin elastomers	HeLa cells	MTT assay	Demonstrate the usefulness of in vitro cytotoxicity studies to improve the response of materials based on polysiloxane-based polyurethane / lignin elastomers.

Table 1.
Overview of in vitro key studies on cytotoxicity induced by xenobiotics using different assay.

Clonogenic assay

This assay enables assessing whether a cell is capable of dividing and forming a colony after being exposed to a treatment [39]. A cell survival curve defines the relationship between the dose of the agent used and the fraction of cells that retain their ability to reproduce. Cell lines of various origins, neoplastic or normal, of human or rodent origin can be used [19, 39].

This test is considered an extremely useful tool owing to its advantages of low cost, reproducibility, and simplicity. It has been used for several decades to evaluate the effects of radiation, chemotherapy, drug development, drug screening, toxicology, and pharmacology [19, 40].

Sulforhodamine B

Sulforhodamine B (SRB) is a bright pink aminoxanthan dye with two negatively charged sulfonic groups –SO_3– capable of electrostatically binding to cations [41]. Under acidic conditions (when dissolved in 1% acetic acid), SRB increases its affinity for the basic amino acids of proteins and binds selectively to them, providing an index of the cellular protein content if the cells were previously fixed with trichloroacetic acid. After removing the unfixed dye, the dye bound to the viable cells is extracted with alkaline medium (Tris solution, pH 10.5) and the absorbance is read at 564 nm [41, 42].

LDH release colorimetric assay

This assay allows the measurement of LDH enzyme activity using a cocktail of reagents containing lactate, NAD+, diaphrose, and the tetrazolium salt INT [43]. LDH catalyzes the reduction of NAD+ to NADH in the presence of L-lactate, and the formation of NADH can be measured by a coupled reaction, in which the

tetrazolium salt INT is reduced to a red formazan product that can be measured spectrophotometrically [44]. The increase in the LDH activity in the culture supernatant is proportional to the number of cells lysed [43, 44].

Annexin V/PI staining

Annexin V is a recombinant protein that specifically binds to phosphatidylserine residues, which are exposed on the outer surface of the plasma membrane, and is an effective biomarker in apoptotic cells [45]. Annexin V can be combined with a DNA marker that is not membrane-permeable unless the membrane is compromised in order to distinguish apoptotic cells from necrotic cells [45].

It has been reported that the combination of annexin V-FITC and the cationic marker PI can guarantee this differentiation, registering non-apoptotic cells (annexin V-FICT-negative/PI negative), the cells in early apoptosis (annexin V-FICT positive/PI negative) and necrotic cells (annexin V-FICT positive/PI positive). The samples are analyzed in a cytometer providing an objective and fast quantification [46].

TUNEL assay

A useful method to study apoptosis is the TdT-mediated dUTP-biotin nick end-labeling (TUNEL assay). During apoptosis, nuclear endonucleases digest genomic DNA into oligonucleosomal fragments of approximately 180–200 base pairs. DNA fragments are labeled by the catalytic incorporation of labeled 16-dUTP at the free ends by means of the enzyme terminal deoxynucleotidyl transferase (TdT) [47]. The accessibility of the enzyme to the DNA break points is decreased due to nuclear proteins along with the processes of fixation of the sample and the subsequent fixation with ethanol [48]. The signal increases with a greater number of breaks in the DNA chain, and it can be conducted both by flow cytometry and fluorescence. The TUNEL assay has been widely applied in different types of cells to detect DNA damage produced by different types of xenobiotics [49, 50].

Kenacid blue binding assay

Through this assay, the change in total protein content is measured, which is a reflection of cell proliferation. If a compound is cytotoxic to the cells, it must affect at least one or more processes involved in cell proliferation, such as DNA synthesis, the proper functioning of organelles such as mitochondria and lysosomes or affect the integrity of the membrane or protein synthesis [51]. When the cell growth is affected, the number of cells present in the treated culture must be reduced with respect to the control, such that the measurement of the concentration of proteins present in the culture constitutes an index of toxicity [51]. Generally, the cells are exposed to the product for evaluation for a time period of 72 h, and the product is then removed and the cells are exposed to the dye, which then binds to the cellular proteins. Finally, the amount of kenacid blue retained by the cells is determined and the percent of inhibition of cell growth is quantified [51, 52].

Resazurin binding assay

Resazurin (7-hydroxy-3H-phenoxazin-3-one 10-oxide) is a non-fluorescent blue dye that is commonly used for the measurements of cell viability [53]. Resazurin is reduced to resofurin (a highly fluorescent pink dye) by oxidoreductases detected primarily in the mitochondria of viable cells. Resofurin is excreted into the medium, allowing continuous monitoring of the proliferation and/or cytotoxicity of the substances in human cells, animals, bacteria, and even fungi [54]. This dye is not extremely toxic to cells and allows the continuity of studies in the same cells, which saves time and money, especially in the primary cultures where the cells are extremely scarce and valuable. Furthermore, it is sensitive and highly reproducible. It is therefore possible to determine in samples at 530–580 nm as excitation wavelengths and 570–620 nm as emission wavelengths, since this dye has both chromophoric and fluorophore properties [53, 54].

Biomarkers

The term "biomarker" is applied to measure an interaction between the biological system and a chemical, physical, or biological agent, which was evaluated as a functional or physiological response that occurs at the cellular or molecular level and is associated with the probability of the development of a disease [55].

The main objectives with the use of biomarkers in human and environmental toxicology are to measure the exposure to xenobiotic agents that causes diseases and to predict the toxic response that could possibly occur [55]. This approach allowed an increase in the requirement of regulation for the development of drugs, pesticides, and other compounds that can produce adverse effects on the human health in addition to greater impacts on occupational health [55, 56]. Short- and long-term toxicity studies in vitro systems and in experimental animals are very valuable to demonstrate the association of different substances with the appearance of cytotoxicity and the development of mutagenesis, carcinogenesis, and teratogenesis in order to promote early actions for the protection of human health [56].

4. Cytotoxic drugs

The absorption of a drug depends on its physicochemical properties, its formulation, and its route of administration. A drug must cross several semi-permeable cell barriers before reaching the systemic circulation. The cell membranes act as biological barriers that selectively inhibit the flow of drug molecules [57, 58].

Within toxic events that can compromise cell functions by xenobiotic agents such as nanoparticle-based drugs, cellular oxidative stress is an important biological process that must be considered [59]. Oxidative stress is manifested by the production of reactive oxygen species (ROS). ROS are highly reactive, very small molecules that are produced as a result of the presence of an unpaired valence electron shell; they are highly reactive and possess the ability to interact with macromolecules such as lipids, proteins, and DNA. There are various cellular signals that are conveyed through binding to antioxidant response elements or in response to electrophiles, which regulate the expression and coordinate different genes related to their chemoprotective and detoxifying capacities [59, 60].

Determining cell viability is essential when analyzing the efficacy of a new drug or treatment [57]. Not all drugs have the same underlying mechanism or the same level of effect, therefore analyzing how they affect cell health can be a key indicator of whether the drugs may work for a specific intended result. The pharmaceutical industry uses a variety of cytotoxicity tests to screen compositions [57]. Chemicals, drugs, and pesticides all affect human cells in different ways, and these tests can uncover the exact mechanisms of how these xenobiotics work in the human body. Cytotoxicity assays can uncover processes such as the destruction of cell membranes, irreversible binding to receptors, impaired protein synthesis, irreversible binding to receptors, and others [19].

Cytotoxic drugs are preferably used to treat neoplastic diseases, these include DNA alkylating agents [61], antimetabolites [62], and microtubule-active agents [63], topoisomerase inhibitors [64], among others. The goal of the pharmaceutical industry is to create increasingly efficient cytotoxic drugs for cancer treatment, with specific targets for certain cellular targets. Nevertheless, they are drugs with high toxicity, mainly utilized for hematopoietic, renal, hepatic, digestive, and dermal ailments [65, 66].

5. Medical devices

According to the WHO, medical devices refer to any instrument, device, implement, machine, implant, reagent for *in vitro* use, software, material, or other similar or related article. These devices must undergo rigorous tests to determine their biocompatibility when they come into contact with the body, regardless of its mechanical, physical, and chemical properties [67].

With the continuous development of science and technology, if medical devices are new, they must undergo biocompatibility tests, cytotoxicity, sensitization, intradermal irritation, acute systemic toxicity, and a series of tests before entering a clinical setting to ensure that it is safe for use and effective in humans [68]. These cytotoxicity studies are generally quick, simple, and straightforward, and help eliminate materials that may be harmful to the body and further consider whether they need further analysis and evaluation for their safe and effective use [69].

Polyurethanes (PUs) represent a popular and important part of industrial products that are characterized by good flexibility properties, high impact resistance, and durability; these characteristics make them polymers with multiple applications [70]. Their block copolymer character provides them a wide versatility in terms of adapting their physical properties and compatibility; thus, PUs are interesting for internal uses (*in vivo*), especially in short-term applications, such as in catheters or implants. Similarly, they are interesting for external use applications *(in vitro*), such as controlled drug release systems. The PUs used as biomedical materials must comply with the mechanical properties for the intended application and must be non-toxic, biodegradable according to the function to be fulfilled, and biocompatible [69, 70].

Biodegradable polymers have gained much attention presently in the medical field in the search for new materials for treating health problems that arise due to their attractive physical properties and good biocompatibility [71].

6. Pesticides

Pesticides, especially herbicides that are used routinely in crop production, have been shown to cause detrimental effects on the human health [72]. These compounds are easily absorbed via different routes, such as the gastrointestinal and respiratory tracts and through the skin. Due to their high stability and affinity for adipose tissues, they can be metabolized and stored in the human organs, mainly in the adipose tissues [72]. Several human diseases have been associated with pesticide exposure, including cancer, hypertension, neurodegenerative diseases (Parkinson's), and diabetes [73–76]. Due to the toxicity and extreme persistence of pesticides in the environment, different studies have been conducted on the toxicity of these agents [77].

Glyphosate for example is a broad spectrum post-emergent herbicide used in both agricultural and non-agricultural areas for weed control [78, 79]. Within the investigations in this regard, Nagy et al. [80] compared the cytotoxic and genotoxic potentials of the active ingredient glyphosate using mononuclear white blood cells that were treated with different concentrations of glyphosate and others with three glyphosate-based herbicides and found that glyphosate induces significant cytotoxicity and genotoxicity effects [80].

The cell metabolic activity is an important indicator of cell viability, and succinate dehydrogenase is an enzyme complex found in the inner mitochondrial membrane of eukaryotic cells that can be used to reflect the viability of these cells [81].

In this field, Devi et al. [82] used rotenone and chlorpyrifos to evaluate the interaction of these pesticides with the protein malate dehydrogenase (MDH) and the consequent cytotoxicity induced by these pesticides. The authors found that rotenone and chlorpyrifos bind strongly to MDH, interfering with protein folding and triggering alterations in their secondary structure.

In this sense, it has also been shown that exposure to pesticides can induce oxidative DNA damage, single and double breaks, and adduct formation [83]. Although there are different DNA repair mechanisms for these damages and for the maintenance of cellular integrity, excessive damage or irreparable damage can lead to cell death processes [8, 83, 84].

In fact, it has been described that some pesticides induce cell death through apoptosis signaling pathways in order to maintain cell homeostasis [85, 86]. Pesticide-induced apoptosis can form the basis of several human diseases, such as cancer and neurological diseases [87]. For this reason, studies in this field have been consistently increasing.

7. Radiation

Radiation, according to its energy, can be classified into ionizing and non-ionizing types [88]. In this sense, ionizing radiation corresponds to the radiation of higher energy (shorter wavelength) within the electromagnetic spectrum. These radiations have sufficient energy to remove electrons from the atoms with which they interact to produce ionizations, while the non-ionizing radiations are those

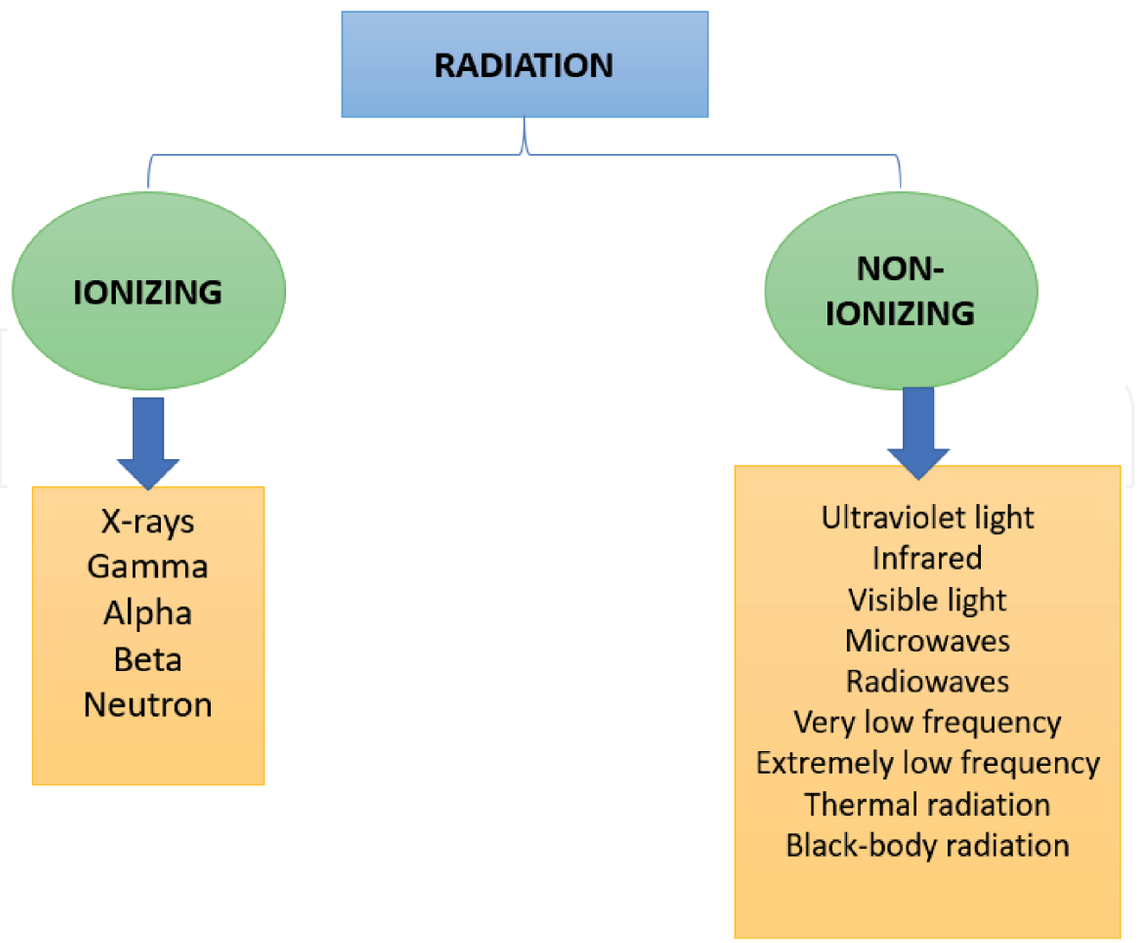

Figure 1.
Types of radiation.

that do not have sufficient energy to remove an electron from the atom they interact with, that is, they do not produce ionizations [88]. **Figure 1** illustrates the types of radiation.

Ionizing radiation is considered to be more dangerous because it has sufficient energy to alter matter (through ionizing energy). Depending on the environment in which the radiation collides, the mechanism of action of the radiation differs. These types of action mechanism can be classified into a direct-action mechanism, which consists of transferring energy to molecules such as proteins, lipids, or DNA, among others, which causes the bond to break down and, consequently, cause damage to the cells. The indirect mechanism of action occurs when energy is absorbed by water molecules in the body and, consequently, radiolysis occurs. In this process, the free radicals of OH^- and the release of H^+ ions are produced, which recombine and can produce H_2, H_2O, and H_2O_2, which when reacting with molecules such as glucose and cholesterol, among others, can cause damage momentary in the metabolism and if they react with DNA, structural damage is generated [89, 90].

These radiations cause DNA damage in different ways, such as double chain breaks, single chain breaks (SSB), hydrogen bond breakage, base dimers, DNA–DNA cross-linking, DNA-protein cross-linking, loss of bases, base modification, and alteration and damage of the repair mechanisms by interaction with cell cycle proteins such as cyclins CDKs and p53 regulator of cell apoptosis, all of which lead to mutations or structural abnormalities that increase the genomic instability [89–91].

8. Conclusions

The damage at the cellular level, either in some structures or in processes that affect cell maintenance, division, or survival, can lead to processes of cell death. An important point in drug evaluation is to provide an alternative approach to improve the predictive capacity of cytotoxicity assays based on cell analysis through incorporation of more specific parameters and/or more appropriate cellular systems. By means of this approach, cytotoxicity can be defined in an integrated manner, based on genomic, proteomic, and cytomic data, starting from the molecules to cells and from the cells to tissues.

The advent of new and improved cytotoxicity assays that are safe, robust, and affordable has been instrumental in advancing the pharmaceutical development process. With these analyzes, presently, research has begun to rely less on animal testing and more on studies that are more economical.

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