ROS

REVIEW ARTICLES

# Pesticides as Inducers of Oxidative Stress

**Agata Jabłońska-Trypuć**

Department of Sanitary Biology and Biotechnology, Faculty of Civil Engineering and Environmental Engineering, Bialystok University of Technology, Białystok, Poland

Correspondence: a.jablonska@pb.edu.pl (A.J-T.)

*Jabłońska-Trypuć A. Reactive Oxygen Species 3(8):96–110, 2017; ©2017 Cell Med Press*
*http://dx.doi.org/10.20455/ros.2017.823*
*(Received: January 6, 2017; Revised: January 16, 2017; Accepted: January 17, 2017)*

**ABSTRACT |** Pesticides are a widely used group of substances destined to kill pests; however, the term includes also agents intended for use as plant growth regulators, defoliants, desiccants, and compounds applied to crops before and after harvesting to protect the commodity from deterioration during storage. Pesticides should be toxic only to target organisms and safe for the others; however, they have become an integral part of the ecosystem and are hazardous to animals and people. Although the influence of pesticides on human health has yet to be well studied, literature data already revealed their influence on oxidative stress parameters. The stimulatory effect of pesticides on lipid peroxidation, and protein, sugars and DNA oxidation processes has been documented. The aim of this review is to discuss selected insecticides, herbicides, and fungicides regarding their molecular mechanisms of action, chemical structure, and influence on basic oxidative stress parameters tested in animals and selected cell cultures in vitro.

**KEYWORDS |** Fungicides; Herbicides; Insecticides; Lipid peroxidation; Oxidative stress; Pesticides; Reactive oxygen species

**ABBREVIATIONS |** ARE, antioxidant response element; DDT, dichlorodiphenyltrichloroethane; GSH, reduced form of glutathione; GSSG, oxidized glutathione; HCH, hexachlorocyclohexane; 4-HNE, 4-hydroxynonenal; $LO^{\bullet}$, alkoxyl radical; $LOO^{\bullet}$, peroxyl radical; MDA, malondialdehyde; $MPP^{+}$, 1-methyl-4-phenylpyridinium; NF-κB, nuclear factor kappaB; $O_2^{\bullet-}$, superoxide anion radical; $OH^{\bullet}$, hydroxyl radical;. 8-OH-G, 8-hydroxyguanine; ROS, reactive oxygen species; $RS^{\bullet}$, thiyl radical; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-alpha

**CONTENTS**

## 1. INTRODUCTION

Pesticides are widely known and used for the protection of plants, plant products, and also utilized by various industrial branches. They are recognized as toxic and hazardous compounds. Although very strict controls on their application are implemented, there is still a serious risk that these compounds will contaminate water, soil, and food by spreading into the environment. Statistical data clearly show that the annual consumption of pesticides in years 1993–2003 in European Union did not decrease [1].

In general, pesticides should be toxic to pests; but on the other hand, they should exhibit a low toxicity towards humans and aquatic organisms. These compounds should have also an appropriate ability to undergo biodegradation so that after the fulfillment of their function they wouldn't be harmful to the environment and the accumulation would not occur. There are many criteria according to which pesticides can be divided into several different groups. Depending on the target organisms, pesticides are classified into zoocides (formulations for controlling animal pests), antimicrobials (formulations for controlling bacteria), herbicides (agents for weed control), fungicides (agents for controlling fungi), and many others. Frequently, based on their chemical structure, a different classification scheme of pesticides is applied. In this regard, pesticides can be classified into nonorganic pesticides (arsenic and fluoride pesticides) and organic pesticides (organochlorine, organophosphorus, carbamates, phenoxyacetic acid derivatives, and triazine derivatives).

A variety of pesticides was commonly used in agricultural programs, which caused significant environmental pollution and consequently health hazards. Literature data have reported cases of severe and chronic toxicity in humans [2–4]. People are constantly exposed to pesticides due to the presence of these compound residues in food and water. Exposure also results from their usage in the food storage. In the European Union, only a part of pesticides is covered by the monitoring program, and studies point to that more than half of vegetables, fruits, and cereals are contaminated with products resulting from the breakdown of pesticides [1, 5–7].

According to the World Health Organization (WHO), pesticide residues in food are defined as a sum of chemical compounds present in food products as a result of pesticides usage. Maximum residue levels of pesticides are defined for certain food products and food ingredients, and the values are expressed as mg/kg of the product. As of September 1, 2008, in all European Union countries, uniform rules concerning acceptable level of pesticides residues in food products are applied. Legal acts relating to pesticides in food have become available on the website (http://ec.europa.eu/food/plant/pesticides/; accessed on February 23, 2017).

In the years 1988—1994, in the United States, studies were conducted to determine the number of people exposed to pesticides and their residues. Obtained results indicated the presence of methyl phosphate, ethyl phosphate, and other metabolites formed during transformations of pesticides in human urine [8]. Such studies weren't conducted in Europe; therefore, the precise number of people exposed to pesticides in this region cannot be determined. As toxic chemicals which have become an integral part of the ecosystem, pesticides are hazardous to non-target organisms, including people; however, their influence on human health has yet to be well studied. Laboratory research aims to explain the mechanisms by which pesticides have an impact on human metabolism (e.g., by inducing an increased level of oxidative stress and free radical generation). Prolonged or increased oxidative stress is detrimental to cells due to its ability to disrupt cellular metabolism. Cell metabolic dysfunctions may lead to permanent changes in the DNA, RNA, protein, lipid, and sugar structures. The consequence of these changes is most often the loss of the biological function followed by, at the level of the whole organism, the development of pathological processes. Oxidative stress, generated also by pesticides and products of their metabolism, is considered a major cause of diseases, such as atherosclerosis, diabetes, cataracts, neurodegenerative diseases, autoimmune diseases, and cancer [9–11].

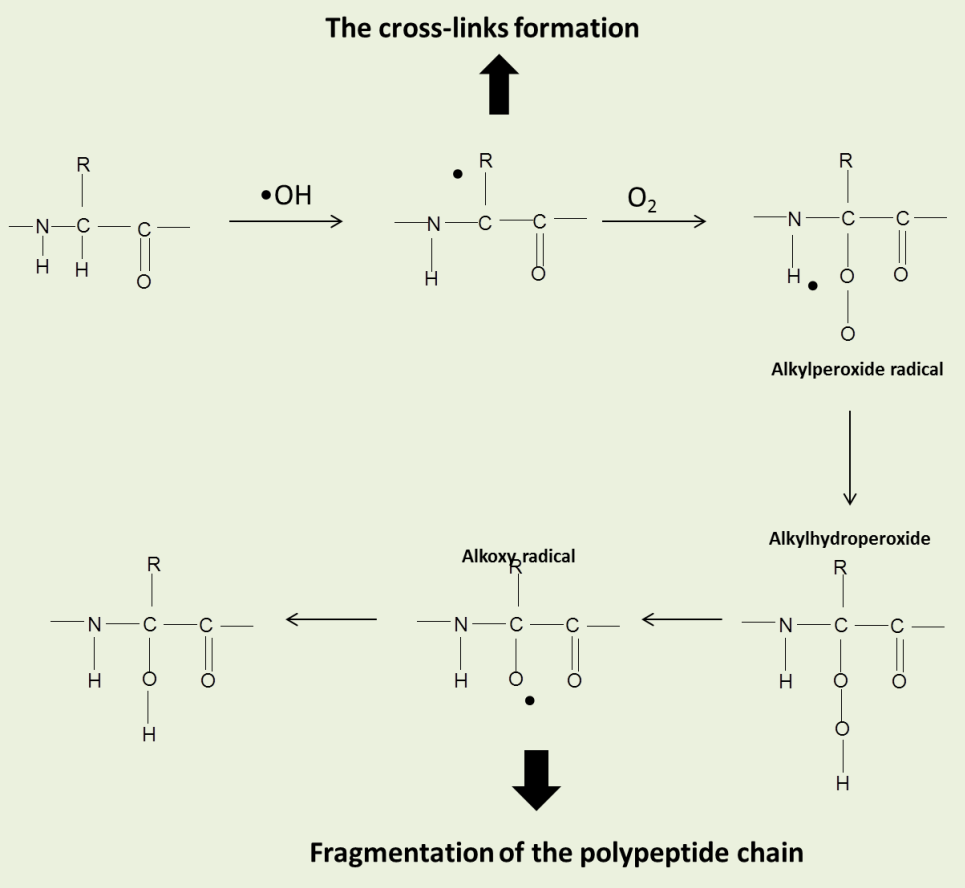


**FIGURE 1. Polypeptide chain oxidation at the α-carbon by reactive oxygen species.** See text (Section 2.1) for detailed description.

## 2. OXIDATIVE STRESS MARKERS

### 2.1. Protein Oxidation

The oxidation of amino acid residues in the polypeptide chain may cause the chain rupture, the creation of cross-linking within the same or other polypeptide chains, as well as the modification of amino acid residues [12]. The examples of amino acids residue oxidation reactions are shown in **Figure 1**. As a result of the above described processes, the biological activity of many proteins may be reduced, increased, modified, or completely lost. Oxidized proteins form aggregates and have an inhibitory effect on the enzymatic systems responsible for their degradation, which in turn promotes the accumulation of modified proteins in cells [13]. The accumulation of oxidized protein products impairs the function of cells and could even result in cell death. During oxidative stress, the oxidation of the cellular thiol groups occurs directly because of the activity of reactive oxygen species (ROS), such as the superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals ($OH^{\bullet}$). The products of the thiol group oxidation are thiyl radicals ($RS^{\bullet}$), which subsequently are dimerized to form disulfides (**Figure 2**). Oxidative damage of thiol groups causes a rapid loss of the protein biological activity, which subsequently leads to significant dysfunction of a number of transporters and enzymes and disruption of calcium homeostasis. The biggest threat to living cells under the influence of oxidative stress is the oxidation of the protein thiol

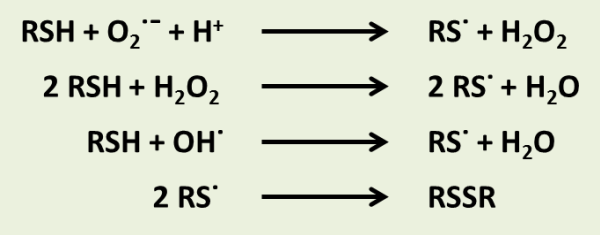


**FIGURE 2. Oxidation of thiol groups by reactive oxygen species.** See text (Section 2.1) for detailed description.

groups in cell membranes. This can lead to the disintegration of the cell membranes and an increase in their permeability. The reactions of ROS with proteins not only cause the protein oxidation, but also the formation of reducing groups in proteins, which subsequently can reduce cytochrome c and metal ions [14, 15].

Protein thiol groups are in equilibrium with the thiol groups of the reduced for of glutathione (GSH), whose main function is to keep the thiol groups of proteins in a reduced state (the reduction of disulfide bridges in many cases is essential for the functional activity of proteins). GSH thiol groups participate in the removal of electrophilic xenobiotics. GSH is considered the most important "thiol buffer" because it regenerates antioxidants, such as vitamin E through the tocopheryl radical reduction [16, 17]. According to the literature, the toxicity of a variety of pesticides, mainly from the group of dithiocarbamates (e.g., zineb, tiuram, disulfiram), is connected with their adverse effect on protein structure. The observed increase in the concentration of protein carbonyl groups supports the above notion [18, 19].

### 2.2. Lipid Peroxidation

One of the most important biological processes connected with the activity of ROS is lipid peroxidation. The cascade process of the oxidation of unsaturated fatty acids present in lipids, in which the peroxide compounds are formed, provides a continuous supply of free radicals and initiates subsequent peroxidation reactions. Peroxidation is a process that occurs mainly with polyunsaturated fatty acid residues, which are part of phospholipids—basic components of cell membranes [20]. Lipid peroxidation occurs in three stages: initiation, propagation, and termination. The initiation phase involves the detachment of a hydrogen atom from a polyunsaturated fatty acid molecule or from this type of fatty acid residues that form part of phospholipid (primary building block of cell membranes). Fatty acids contained in cell membranes are easily subject to free radical attack. Lipid peroxidation may be initiated by a hydroxyl radical (OH$^\cdot$), peroxyl radical (LOO$^\cdot$), alkoxyl radical (LO$^\cdot$), or alkyl radical (L$^\cdot$) (**Figure 3**). It also may be initiated by ozone, nitrogen oxide, nitrogen dioxide, sulfur dioxide, and hypochlorite [21–23].

During the propagation reaction (prolongation), alkyl free radicals react with oxygen to form peroxyl free radicals. These in turn may abstract hydrogen atoms from additional polyunsaturated fatty acids (LH). In such a reaction, the free radical does not disappear, but reacts with another fatty acid molecule. This cycle is repeated many times until the reaction of termination. The termination reaction may occur between two alkyl free radicals, two peroxyl free radicals, or two different radicals that are present in the system. Products of the reaction that occurs in biological membranes are phospholipids dimers. Lipid peroxidation also occurs in the cell membranes which contain proteins, and the free radicals formed during the peroxidation can also react with these proteins. Protein free radicals that participate in the termination reaction and form protein-lipid connections are also formed as a result of lipid peroxidation.

During lipid peroxidation, re-initiation process takes place, in which the lipid peroxides may decompose. This phenomenon can also be caused by transition metals (e.g., Fe and Cu). Further transformation of lipid peroxidation products occur, among others, through β-elimination, which leads to the disintegration of polyunsaturated fatty acid residues and to the formation of several-carbon and even over a dozen-carbon fragments. The end-products of the above reaction are, among others, malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), which may damage the nucleic acid molecules and proteins [24].

As a result of lipid peroxidation, the other products from the aldehyde group are produced, including 4-hydroxyalkenal, 2-alkenal, hepta-2,4-dienal, 5-hydroxyoctanal, and many others, as well as hydrocarbons such as ethane and pentane. Among the above mentioned products of lipid peroxidation, 4-HNE is the most toxic, and MDA is characterized by mutagenic activity in bacterial and mammalian cells

| Propagation Phase of LPO | Termination Phase of LPO |
|---|---|
| 1. $L\bullet + O_2 \rightarrow LOO\bullet$ | 1. $L\bullet + L\bullet \rightarrow L\text{-}L$ |
| 2. $LOO\bullet + LH \rightarrow LOOH + L\bullet$ | 2. $LOO\bullet + LOO\bullet \rightarrow L{=}O + LOH + O_2$ |
| | 3. $LOO\bullet + L\bullet \rightarrow L{=}O + LOH$ |

**FIGURE 3. Propagation and termination phase reactions during lipid peroxidation (LPO) process.** See text (Section 2.2) for detailed description.

and carcinogenic activity in rats. Elevated concentration of thiobarbituric acid-reactive compounds reflects an increased lipid peroxidation level, and it is the fundamental parameter used to study lipid peroxidation [25, 26]. Lipid peroxidation reactions undergo intensification in cells exposed to oxidative stress, e.g., during infection, inflammation, and in the processes of aging, neurodegenerative diseases, and cancer [27].

The mechanisms of the cytotoxic effects of some pesticides from the group of dithiocarbamates (e.g., maneb, zineb, and thiram) are associated with the induction of cellular lipid peroxidation process. This process causes cytoplasmic and mitochondrial membrane damage and depolarization, resulting in an increased production of free oxygen radicals within the cells [28].

### 2.3. DNA and Sugar Oxidation

As a result of ROS influence on DNA, numerous oxidative damages, including, among others, damage to the individual nucleotide bases, DNA strand breaks, and the formation of adducts, occur [29]. Hydrogen peroxide and superoxide anion do not influence directly the nucleic acid components. The hydroxyl radical is largely responsible for the oxidative damage to DNA. Hydroxyl radical reactions with nucleic acids result in damage of nitrogenous bases, sugar moieties, or crack formation in nucleic acid strands, and the production of DNA-protein cross-linking [30]. The most susceptible to reaction with the hydroxyl radical are thymidine residues. The resulting thymidine residue free radicals react with oxygen to form the corresponding peroxides (**Figure 4**). There are three thymidine peroxide isomers having a peroxide group in position 5 or 6 of the pyrimidine ring or associated with a methyl group carbon. These are cis-6-hydroxy-5-hydroperoxy-5,6-dihydrothymidine, cis-5-hydroxy-6-hydroperoxy-5,6-dihydrothymidine, and 5-hydroperoxymethyl-2′-deoxyuridine [21]. Another nitrogenous base, which is readily oxidized, is guanine. As a result of the hydroxyl radical reaction with guanine, the 8-hydroxyguanine (8-OH-G), which is the most common mutagenic damage to the DNA molecule, is formed. Due to the above substitution, transversion type mutation occurs, in which the pair of C-G (guanine-cytosine) passes into a pair of A-T (thymine -adenine) [31, 32].

Another type of mutation which arises due to oxidative stress is a transition type of mutation, where cytosine passes into a thymine, which leads to thymine glycol formation. This compound is produced by oxidation of double bonds in the positions 5 and 6 of the methylcytosine ring. Oxidation of the thymine methyl group leads to the formation of 5-hydroxyuracyl, that impacts on the interaction of DNA with a number of transcription factors, which in turn may alter the gene expression. It should be also mentioned that the single and double breaks in DNA strands are produced by the reaction of hydroxyl radical with the deoxyribose in DNA [33].

## 3. PESTICIDES AS INDUCERS OF OXIDATIVE STRESS

Pesticides are one of the main sources of environmental and health problems that cause changes in oxidative stress parameters and are hazardous, endocrine disrupting chemicals. Exposure to pesticides is one of the causes of the increased oxidative stress level, and it may result in altered disease susceptibility. The mechanisms by which pesticides in-

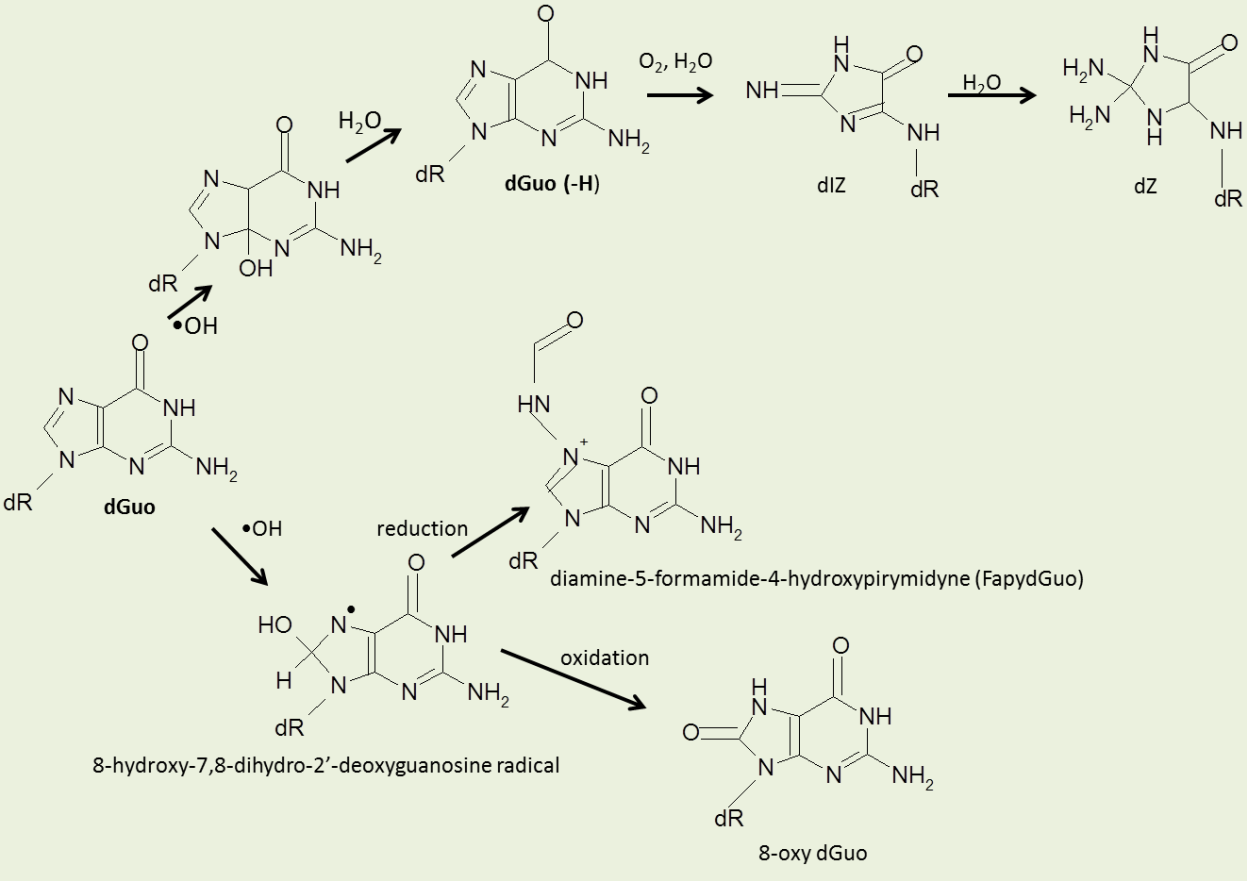


**FIGURE 4. Reactions of hydroxyl radical with guanine, leading to the formation of 8-hydroxyguanine.** See text (Section 2.3) for detailed description.

fluence human metabolism at the cellular level are still not precisely defined. Therefore, it is very important to conduct a variety of studies connected with that phenomenon. The effect of a pesticide usually depends on its chemical structure, dose, and time of exposure.

### 3.1. Insecticides

This group of insecticides is very complex and widespread. The oldest known insecticides, used already in the 1800s, are pyrethrins derived from yellow *Chrysanthemum cinerariifolium* and *Tanacetum cinerariifolium*. Nowadays, a variety of synthetic pyrethroid derivatives with an excellent chemical stability are produced (**Figure 5a**). In general, pyrethroids are safe and exhibit rather low toxicity to mammals and birds as well as good biodegradability; however, extensive use of these compounds causes an increase in the risk of intoxication for non-target species, such as water and soil organisms. According to the literature, pyrethroids induce oxidative stress at the tissue and cell levels [34]. An example of their pro-oxidative activity is the increased content of antioxidant enzymes under the influence of pyrethroids in rat erythrocytes, which can be considered as an adaptation to increased oxidative stress [35].

The other group of insecticides is organophosphates, which have cholinesterase-inhibiting activity and contain phosphorus derived from phosphoric acid (**Figure 5b**). They are the most toxic agents to vertebrates among all pesticides, and their mecha-

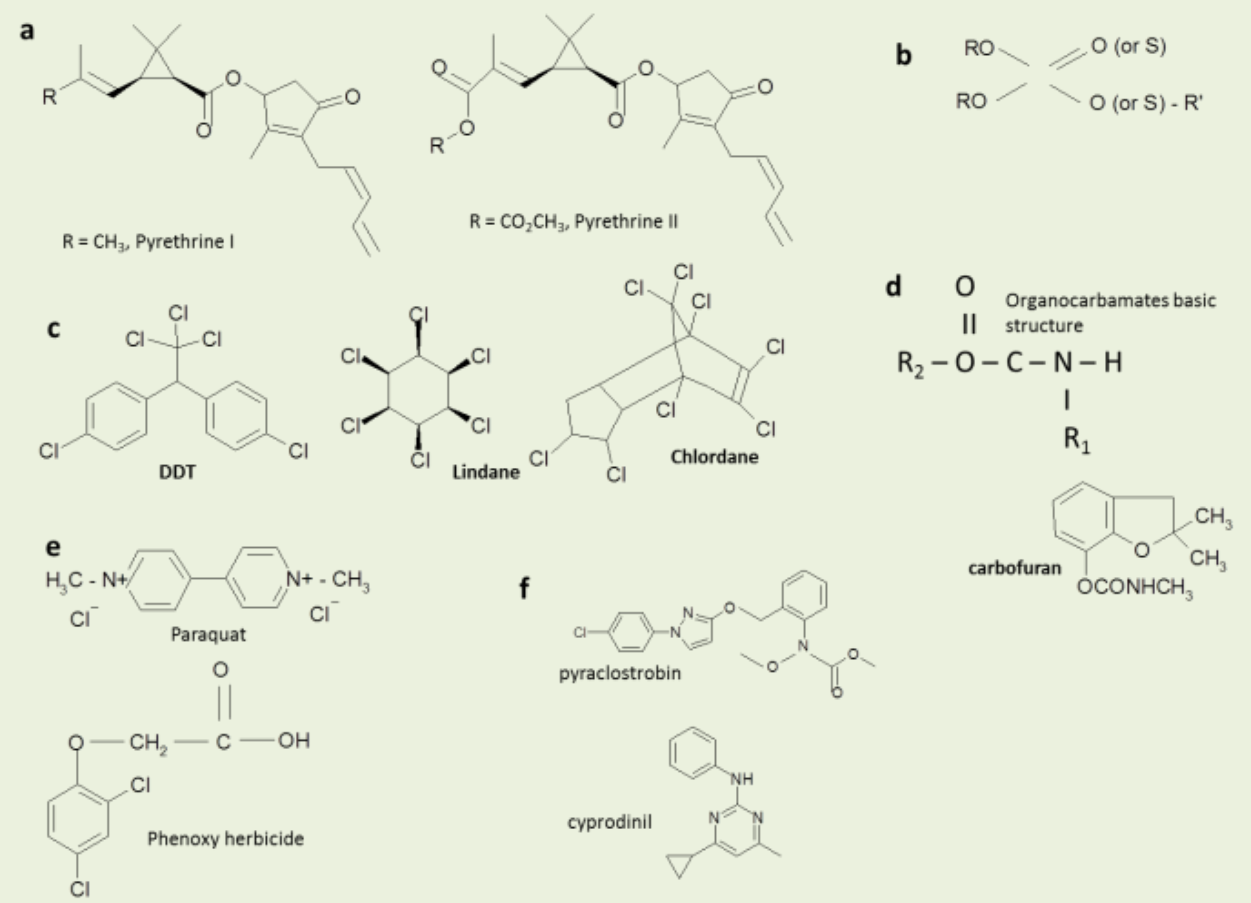


**FIGURE 5. Chemical structures of selected pesticides.** Shown are: (a) pyrethrine compounds; (b) organophosphate compounds; (c) organochlorine compounds; (d) organocarbamate compounds and their derivatives; (e) selected herbicides; and (f) selected fungicides.

nism of action is the inhibition of the function of carboxylic ester hydrolases, e.g., chymotrypsin, acetylcholinesterase, plasma or butyrilcholinesterase, plasma and hepatic carboxylesterases, paraoxonases, and other non-specific esterases. Several studies indicated that organophosphates induce lipid peroxidation, and what follows—acute tubular necrosis, which accompanies organophosphate toxicity, is connected with ROS generation and lipid peroxidation process [36, 37]. Amal et al. reported that organophosphates caused a significant increase in MDA (an end product of lipid peroxidation) level and a decrease in antioxidants (GSH and catalase) content [38]. The MDA level serves as an index of peroxidative damage in different tissues, and GSH, as a substrate in glutathione peroxidase/glutathione reductase system, detoxifies lipid peroxidation products. The decrease in GSH content favors lipid peroxidation and induces oxidative stress [39]. According to Verma et al., chlopyrifos causes a decrease in the level of GSH and an increase in the level of oxidized glutathione (GSSG). Chlorpyrifos has been reported to induce oxidative stress in the kidney, liver, brain, and fetus of the pregnant rats [40].

Organochlorines are a well-known group of insecticides that contain, in their chemical structure, carbon, chlorine, and hydrogen (**Figure 5c**). A very popular one, although banned in 1960s due to its hazards to the environment, is DDT (dichlorodiphenyltrichloroethane). According to the literature, DDT

and its derivatives caused high levels of oxidative stress and lipid peroxidation [41]. Perez-Maldonado et al. reported that DDT may cause apoptosis in human blood mononuclear cells, and Hassoun et al. showed that DDT, lindane, chlordane, and endrin exposure resulted in a significant increase in hepatic lipid peroxidation level and DNA damage [42, 43]. Hexachlorocyclohexane (HCH, lindane) is the only, still widely used organochlorine insecticide which is effective against pests and scabies. The main molecular mechanism by which lindane causes severe tissue damage is lipid peroxidation, which has been confirmed in rat blood, brain, testis, and liver [44–46]. Olgun and Misra reported that malathion and permethrin induce toxicity in immune cells, which results from an elevated level of oxidative stress [47]. Cyclodienes are compounds from the group of organochlorines which also induce oxidative stress and tissue damage in the liver and brain tissue of mice, and examples include endrin (in mice, rats, stouts, guinea pigs), endosulfan, dielderin, and toxafene [48]. Hassoun et al. found out that chlordane is the cause of oxidative tissue damage based on studies that revealed the level of hepatic lipid peroxidation and DNA damage [43].

Organocarbamate pesticides are one of the several classes of insecticides widely used in homes, gardens, and agriculture (**Figure 5d**). The results of a study performed by Elisi et al. demonstrated the effects of four carbamates (aldicarb, aldicarb sulfone, aldicarb sulfoxide, and propoxur) on GSH content and antioxidative enzymes activity in the mammalian cellular model CHO-K1 cells after 24 h exposure. All tested pesticides caused depletion of GSH content, no change in GSSG content, decrease in GSH/GSSG ratio, and the induction of glutathione reductase and glutathione peroxidase activities [49]. Rai et al. demonstrated that carbofuran (another carbamate pesticide) caused an increased level of oxidative stress in rat erythrocytes, which are prone to oxidative damage due to the presence of hemoglobin and polyunsaturated fatty acids in their structure. Increased osmotic fragility of erythrocytes is connected with oxidative stress [50].

### 3.2. Herbicides

In the group of herbicides, very popular in agriculture are bipyridyl herbicides, mainly paraquat and diquat (**Figure 5e**). They are strong poisons, used as contact herbicides and as crop desiccants on products like cotton. In their chemical structure, two pyridine rings can be distinguished, in which one carbon atom is replaced by a nitrogen atom joined by an ethylene group. These compounds are usually produced as salts with chloride ion (paraquat) and bromide ion (diquat). Paraquat, which is more toxic, is also easily absorbed through the respiratory and gastrointestinal tract and skin. On the other hand, diquat's toxicity mainly results from ingestion, because it is poorly absorbed through the intact skin or respiratory system [51].

The mechanism by which pesticides from the bipyridyl group increase an oxidative stress level in the cells is mainly an initiation of the cyclic oxidation/reduction process. The first stage of this process is herbicide's one-electron reduction caused by NADPH, which is one of the main sources of reducing equivalents for the intracellular reduction of paraquat. Free radicals formed in this process donate their electron to oxygen molecule, and as a result of this reaction, superoxide radicals are produced. Following depletion of NADPH, superoxide radicals react with themselves and produce hydrogen peroxide, which can be further concerted to hydroxyl free radicals [52]. Hydroxyl free radicals are very toxic and have the ability to induce lipid peroxidation process. The lung is the major target organ of toxicity, which undergoes a biphasic injury pattern. The consequence of the redox cycle is the destruction of the alveolar epithelium. Subsequently, the next phase occurs in which normal epithelial cells are replaced by fibrous tissue. This leads to pulmonary fibrosis, hypoxemia, and death. The toxic effect of paraquat on lung tissue both in vivo and in vitro is escalated by high oxygen concentration, which indicates the importance of the reaction of paraquat radical with $O_2$ [53]. A study by He et al. revealed that paraquat's main target is mitochondria, where it causes severe damage and an increase in oxidative stress level. Paraquat activity results in lung epithelial cell death, overproduction of profibrogenic cytokines and growth factors, and myofibroblast transformation. The results obtained by He et al. revealed also that cytotoxicity of paraquat is dose-dependent, and it is largely attributed to apoptosis as revealed by caspase 3/7 activation. They also observed a significant increase in ROS production using BEAS-2B cell line as a research model. In conclusion, mitochondrial damage and oxidative stress are the major mecha-

nisms by which paraquat causes fibrosis and lung damage [54].

Rodriguez-Rocha et al. reported that paraquat and $MPP^+$ (1-methyl-4-phenylpyridinium, known as a cyperquat with structural similarity to paraquat) cause the loss of dopaminergic neurons, and this process is mainly connected with oxidative stress. The research models in this study are neuroblastoma cells (SK-N-SH) and human IMR-32 neuroblastoma cells. Regarding paraquat, it was shown that overexpression of manganese superoxide dismutase (MnSOD), but not CuZnSOD, inhibited the oxidative stress and cell death caused by paraquat. On the other hand, oxidative stress induced by $MPP^+$ was insensitive to MnSOD and CuZnSOD overexpression. These results suggest that different mechanisms are involved in the toxicity of selected herbicides [55].

According to de Liz Olivera Cavalli et al., glyphosate, which is a broad-spectrum systemic herbicide, causes oxidative stress and activates a variety of stress-response pathways leading to Sertoli cell death in prepubertal rat testis [56]. Two commonly used phenoxyherbicides, namely, sodium salt of 2,4-dichlorophenoxyacetic acid (2,4-D-Na) and sodium salt of 4-chloro-2-methylphenoxyacetic acid (MCPA-Na) were examined by Bukowska et al. They used human erythrocytes as a research model because of their structural and functional simplicity. Obtained results revealed that the pro-oxidative action of phenoxyherbicides is strongly dependent on the localization of the substituent in the phenol ring of the herbicides [57].

### 3.3. Fungicides

In humans constantly exposed to fungicides from the group of dithiocarbamates, symptoms typical for Parkinson's disease were observed (**Figure 5f**) [58, 59]. This applies in particular to farmers having, in their work, continuous contact with maneb. Maneb in its molecule comprises a manganese atom, and according to the literature, this pesticide is associated with the Parkinson's disease development [60]. The causes and mechanisms of neurodegeneration in this disease are still being unexplained, but it calls attention to the important role of oxidative stress. Maneb is a compound that interferes with the mitochondrial respiratory chain. Mitochondrial dysfunction is associated with the generation of free radicals, which consequently leads to an imbalance in cell redox state [61]. Regueiro et al. tested nine selected fungicides: ametoctradin, boscalid, cyazofamid, dimethomorph, fenhexamid, kresoxim-methyl, mepanipyrim, metrafenoneand, and pyraclostrobin. The results of their study revealed that all these compounds caused a reduction in cell viability and were cytotoxic. The study was performed with the use of primary cultures of cortical neurons derived from cerebral cortices of mouse fetuses. All tested fungicides caused an inhibition of the mitochondrial respiration complex III, an inhibition of the natural electron transport, ATP depletion, and depolarization of the mitochondrial membrane, leading to the increase in cytosolic calcium level and cell death [62]. According to Coleman et al., fungicides such as pyrimethanil, cyprodinil, and fludioxonil caused decreased cellular energy-generating capability, and they influenced the expression of key protective antioxidative enzymes, such as SOD and glutathione peroxidase. The study was performed on U251 and SH-SY5Y cells, which are representative of human central nervous system glial and neuronal cells, respectively [63].

### 3.4. Major Molecular Pathways Involved in Pesticide-Mediated Oxidative Damage

In the toxicological effects of pesticides, induced by oxidative stress, a variety of biological responses are involved. In many different cell signaling pathways, changes in gene expression, stimulation, and/or inhibition of selected signal transduction occur. The role of selected pesticide-mediated oxidative stress in the induction of different cell signaling pathways has been studied mostly in vitro by using an appropriate cell line, but also in vivo in animal research models.

Permethrin is one of the frequently used insecticides from the group of pyrethrins, which decreases the antioxidant defense system and causes major damage to important cellular macromolecules, such as lipids, DNA, and proteins [64]. Oxidative stress-mediated activity of permethrin is shown in **Figure 6**.

An elevated level of oxidative stress, which results from the ROS generation, usually stimulates a variety of cell signaling pathways connected with apoptosis. One of the major cellular mechanisms of defense against oxidative stress is Keap1/Nrf2/ARE pathway. After exceeding a certain level of ROS within the cytoplasm, Nrf2, which is a basic leucine zipper transcription factor, dissociates from Keap1 and is

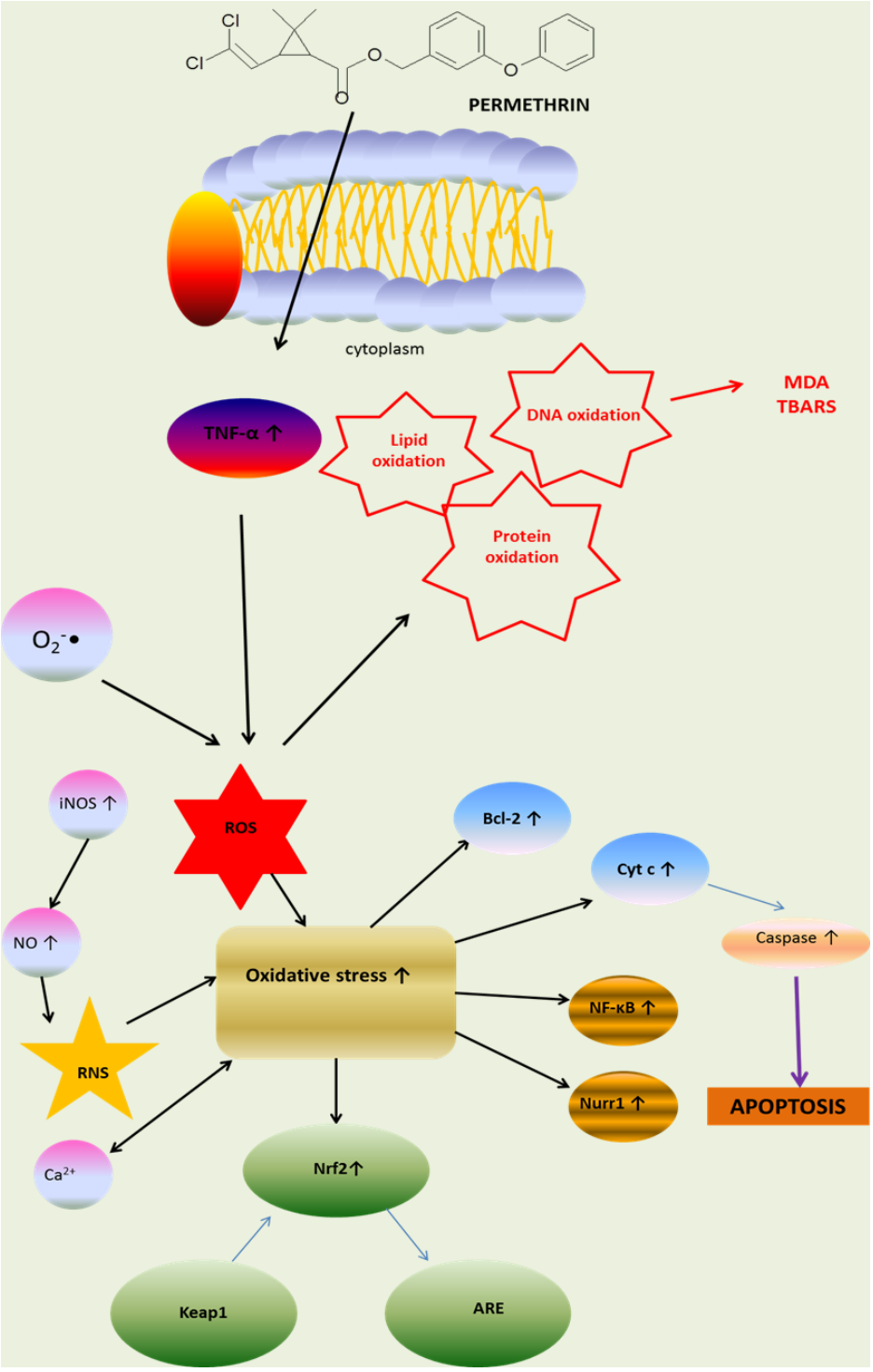


**FIGURE 6. Molecular mechanisms of selected pesticide (permethrin)-induced toxicity.** As depicted, high amounts of ROS and reactive nitrogen species (RNS) induce lipid, protein, and DNA oxidation, leading to apoptosis via different signal transduction pathways.

transferred into the nucleus. Subsequently, it dimerizes with small Maf-binding proteins and binds to ARE (antioxidant response elements), which activates ARE-dependent gene expression. In the next stage, antioxidative enzymes, which protect the cells from ROS overproduction, are activated. It should be also mentioned that $Ca^{2+}$ ions play an important role in cell signaling, apoptosis, cell proliferation, and other oxidative stress-mediated processes [65–67]. Studies conducted on rats revealed that permethrin may cause a significant increase in Nrf2 gene expression and an elevated influx of the intracellular $Ca^{2+}$. These results show that permethrin may act through the activation of the Keap1/Nrf2/ARE pathway [68].

Another mechanism by which permethrin may cause serious damage to the cell macromolecules and lead even to cell death is the mitochondrial apoptotic pathway. The main mitochondrial integrity regulators that participate in this pathway are Bcl-2 and Bax, which belong to the Bcl-2 family. They also influence cytochrome c release and caspase activation. The major function of Bcl-2 is to prevent apoptosis by its antioxidative activity. After mitochondrial damage, Bax is translocated from the cytosol to the mitochondria, and a significant decrease in Bcl-2 expression also occurs. Due to ROS exceeding a certain level, a critical apoptotic event, which is the release of mitochondrial cytochrome c into the cytoplasm, occurs [69, 70]. According to the literature data, exposure of rats to permethrin and its derivatives caused a significant release of mitochondrial cytochrome c, what confirms the existence of mitochondrial apoptotic pathway in pesticide-induced oxidative stress [71].

Taking into consideration of existing evidence, the death receptor pathway should be also mentioned as one of the possible oxidative stress-induced mechanisms. Tumor necrosis factor receptor (TNFR) responsible for the effects of tumor necrosis factor alpha (TNF-α) activates death receptor pathway after the ligation process. This process leads to the activation of caspase-8 which in turn cleaves effector caspase-3. The increased level of TNF-α may cause an increase in ROS generation, as observed in rats exposed to permethrin [72–74]. A transcription factor that belongs to the NR4A family of proteins is Nurr1. It plays an important role in the metabolism of dopaminergic neurons. Its anti-inflammatory influence results from the inhibitory activity towards the transcription factor NF-κB in the brain tissue [75]. Lipid peroxidation products influence Nurr1 expression, and an increased level of oxidative stress is one of the causes of Nurr1 cytosolic accumulation and NF-κB induction. Exposure of rats to permethrin induces an increase in the expression of pro-inflammatory NF-κB transcription factor and a decrease in Nurr1 gene expression at the same time [68]. These results indicate that Nurr1 and NF-κB pathways may be responsible for the mechanisms related to oxidative stress caused by pesticides.

Another widely used pesticide in agriculture is paraquat which interferes with the intracellular electron transfer photosystems. After entering the cell, it acts as a redox cycling compound which causes mitochondrial toxicity. It was shown that NADPH oxidase, nitric oxide synthase, and NADPH-cytochrome P450 reductase initiate the redox cycling of paraquat in plasma membranes and cytosolic compartments. NADPH-cytochrome P450 reductase catalyzes the reduction of paraquat to the monocation radical $PQ^{\bullet+}$. This radical reaction with an oxygen molecule causes the generation of $O_2^{\bullet-}$ and the regeneration of paraquat dication, which subsequently may undergo the reduction-oxidation cycle. All the enzymes involved in paraquat metabolism within the cell are NADPH-dependent, which in turns has an impact on the glutathione disulfide recycling to GSH. Similarly to permethrin, paraquat also causes the release of the cytochrome c to the cytosol and influences the expression of Bcl-2 and Bax [76].

## 4. CONCLUSIONS

Owing to diverse chemical structures, biological activity, and indiscriminate application of pesticides in the environment, the problem of their hazardous side effects has arisen. As described above, the results of studies conducted in animals and a variety of human cell lines have proved that these compounds increase oxidative stress level, which leads to the development of a variety of disease processes, including cancer. There is also a growing body of evidence suggesting that certain selected pesticides are neurotoxic and increase the risk of Parkinson's disease, likely via a mechanism involving oxidative stress. Therefore, the use of pesticides should be limited and monitored, considering their negative influence on human health.

**ACKNOWLEDGMENTS**

This work is financially supported by project number S/WBiIŚ/3/2015.

**REFERENCES**

1. Bjorling-Poulsen M, Andersen HR, Grandjean P. Potential developmental neurotoxicity of pesticides used in Europe. *Environ Health* 2008; 7:50. doi: 10.1186/1476-069X-7-50.
2. Abdollahi M, Jalali N, Sabzevari O, Hoseini R, Ghanea T. A retrospective study of poisoning in Tehran. *J Toxicol Clin Toxicol* 1997; 35(4):387–393.
3. Jalali N, Pajoumand A, Abdollahi M, Shadnia S. Epidemiological survey of poisoning mortality in Teheran during 1997–1998. *Toxicol Lett* 2000; Suppl. 1(116):309.
4. Abdollahani M, Jalali N, Sabzevari O. Pesticide poisoning during an 18-month period (1995–1997) in Teheran, Iran. *Irn J Med Sci* 1999; 24:77–81.
5. Costa LG, Giordano G, Guizzetti M, Vitalone A. Neurotoxicity of pesticides: a brief review. *Front Biosci* 2008; 13:1240–1249.
6. Ray DE. Pesticide neurotoxicity in Europe: real risks and perceived risks. *Neurotoxicology* 2000; 21(1–2):219–221.
7. Burns CJ, McIntosh LJ, Mink PJ, Jurek AM, Li AA. Pesticide exposure and neurodevelopmental outcomes: review of the epidemiologic and animal studies. *J Toxicol Environ Health B Crit Rev* 2013; 16(3–4):127–283. doi: 10.1080/10937404.2013.783383.
8. Mage DT, Allen RH, Gondy G, Smith W, Barr DB, Needham LL. Estimating pesticide dose from urinary pesticide concentration data by creatinine correction in the Third National Health and Nutrition Examination Survey (NHANES-III). *J Expo Anal Environ Epidemiol* 2004; 14(6):457–465. doi: 10.1038/sj.jea.7500343.
9. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. Pesticides and oxidative stress: a review. *Med Sci Monit* 2004; 10(6):RA141–147.
10. Etemadi-Aleagha A, Akhgari M, Abdollahi M. A brief review on oxidative stress and cardiac diseases. *Mid East Pharmac* 2002; 10:8–9.
11. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007; 39(1):44–84. doi: 10.1016/j.biocel.2006.07.001.
12. Stadtman ER, Levine RL. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids* 2003; 25(3–4):207–218. doi: 10.1007/s00726-003-0011-2.
13. Grune T, Davies KJ. The proteasomal system and HNE-modified proteins. *Mol Aspects Med* 2003; 24(4–5):195–204.
14. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. *Clin Chim Acta* 2003; 329(1–2):23–38.
15. Dalle-Donne I, Aldini G, Carini M, Colombo R, Rossi R, Milzani A. Protein carbonylation, cellular dysfunction, and disease progression. *J Cell Mol Med* 2006; 10(2):389–406.
16. Balcerczyk A, Bartosz G. Thiols are main determinants of total antioxidant capacity of cellular homogenates. *Free Radic Res* 2003; 37(5):537–541.
17. Balcerczyk A, Grzelak A, Janaszewska A, Jakubowski W, Koziol S, Marszalek M, et al. Thiols as major determinants of the total antioxidant capacity. *Biofactors* 2003; 17(1–4):75–82.
18. Astiz M, de Alaniz MJ, Marra CA. Antioxidant defense system in rats simultaneously intoxicated with agrochemicals. *Environ Toxicol Pharmacol* 2009; 28(3):465–473. doi: 10.1016/j.etap.2009.07.009.
19. Grosicka E, Sadurska B, Szumilo M, Grzela T, Lazarczyk P, Niderla-Bielinska J, et al. Effect of glutathione depletion on apoptosis induced by thiram in Chinese hamster fibroblasts. *Int Immunopharmacol* 2005; 5(13–14):1945–1956. doi: 10.1016/j.intimp.2005.06.017.
20. Marnett LJ. Oxy radicals, lipid peroxidation and DNA damage. *Toxicology* 2002; 181–182:219–222.
21. Bartosz G. Peroxynitrite: mediator of the toxic action of nitric oxide. *Acta Biochim Pol* 1996; 43(4):645–659.
22. Agha AM, Gad MZ. Lipid peroxidation and lysosomal integrity in different inflammatory models in rats: the effects of indomethacin and

naftazone. *Pharmacol Res* 1995; 32(5):279–285.
23. Banerjee BD, Seth V, Bhattacharya A, Pasha ST, Chakraborty AK. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. *Toxicol Lett* 1999; 107(1–3):33–47.
24. Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE, Marnett LJ. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. *J Biol Chem* 2003; 278(33):31426–31433. doi: 10.1074/jbc.M212549200.
25. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 2006; 160(1):1–40. doi: 10.1016/j.cbi.2005.12.009.
26. Zarkovic N. 4-hydroxynonenal as a bioactive marker of pathophysiological processes. *Mol Aspects Med* 2003; 24(4–5):281–291.
27. Niki E, Yoshida Y, Saito Y, Noguchi N. Lipid peroxidation: mechanisms, inhibition, and biological effects. *Biochem Biophys Res Commun* 2005; 338(1):668–676. doi: 10.1016/j.bbrc.2005.08.072.
28. Kang D, Hamasaki N. Mitochondrial oxidative stress and mitochondrial DNA. *Clin Chem Lab Med* 2003; 41(10):1281–1288. doi: 10.1515/CCLM.2003.195.
29. Cadet J, Douki T, Gasparutto D, Ravanat JL. Oxidative damage to DNA: formation, measurement and biochemical features. *Mutat Res* 2003; 531(1–2):5–23.
30. Williams GM, Jeffrey AM. Oxidative DNA damage: endogenous and chemically induced. *Regul Toxicol Pharmacol* 2000; 32(3):283–292. doi: 10.1006/rtph.2000.1433.
31. Hsu GW, Ober M, Carell T, Beese LS. Error-prone replication of oxidatively damaged DNA by a high-fidelity DNA polymerase. *Nature* 2004; 431(7005):217–221. doi: 10.1038/nature02908.
32. Lindahl T. Instability and decay of the primary structure of DNA. *Nature* 1993; 362(6422):709–715. doi: 10.1038/362709a0.
33. Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J* 2003; 17(10):1195–1214. doi: 10.1096/fj.02-0752rev.
34. Gabbianelli R, Falcioni G, Nasuti C, Cantalamessa F. Cypermethrin-induced plasma membrane perturbation on erythrocytes from rats: reduction of fluidity in the hydrophobic core and in glutathione peroxidase activity. *Toxicology* 2002; 175(1–3):91–101.
35. Kale M, Rathore N, John S, Bhatnagar D. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. *Toxicol Lett* 1999; 105(3):197–205.
36. Gupta RC, Milatovic D, Dettbarn WD. Depletion of energy metabolites following acetylcholinesterase inhibitor-induced status epilepticus: protection by antioxidants. *Neurotoxicology* 2001; 22(2):271–282.
37. Poovala VS, Huang H, Salahudeen AK. Role of reactive oxygen metabolites in organophosphate-bidrin-induced renal tubular cytotoxicity. *J Am Soc Nephrol* 1999; 10(8):1746–1752.
38. Amal AM, IIowaida AN, Gihan MS, Manal H, Abdel A. Role of oxidative stress and apoptosis into acute organophosphorus intoxicated patient. *J Med Res Inst* 2005; 26:255–263.
39. Maellaro E, Casini AF, Del Bello B, Comporti M. Lipid peroxidation and antioxidant systems in the liver injury produced by glutathione depleting agents. *Biochem Pharmacol* 1990; 39(10):1513–1521.
40. Radhey SV, Anugya M, Nalini S. In vivo chlorpyrifos induced oxidative stress: Attenuation by antioxidant vitamins. *Pesticide Biochem Physiol* 2007; 88:191–196.
41. Gultekin F, Ozturk M, Akdogan M. The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (in vitro). *Arch Toxicol* 2000; 74(9):533–538.
42. Perez-Maldonado IN, Herrera C, Batres LE, Gonzalez-Amaro R, Diaz-Barriga F, Yanez L. DDT-induced oxidative damage in human blood mononuclear cells. *Environ Res* 2005; 98(2):177–184. doi: 10.1016/j.envres.2004.11.001.
43. Hassoun E, Bagchi M, Bagchi D, Stohs SJ. Comparative studies on lipid peroxidation and DNA-single strand breaks induced by lindane, DDT, chlordane and endrin in rats. *Comp Biochem Physiol C* 1993; 104(3):427–431.
44. Simon-Giavarotti KA, Giavarotti L, Gomes LF, Lima AF, Veridiano AM, Garcia EA, et al. Enhancement of lindane-induced liver oxidative stress and hepatotoxicity by thyroid hormone is

reduced by gadolinium chloride. *Free Radic Res* 2002; 36(10):1033–1039.
45. Sahoo A, Chainy GB. Acute hexachlorocyclohexane-induced oxidative stress in rat cerebral hemisphere. *Neurochem Res* 1998; 23(8):1079–1084.
46. Sahoo A, Samanta L, Chainy GB. Mediation of oxidative stress in HCH-induced neurotoxicity in rat. *Arch Environ Contam Toxicol* 2000; 39(1):7–12.
47. Olgun S, Misra HP. Pesticides induced oxidative stress in thymocytes. *Mol Cell Biochem* 2006; 290(1–2):137–144. doi: 10.1007/s11010-006-9178-7.
48. Bagchi D, Hassoun EA, Bagchi M, Stohs SJ. Protective effects of antioxidants against endrin-induced hepatic lipid peroxidation, DNA damage, and excretion of urinary lipid metabolites. *Free Radic Biol Med* 1993; 15(2):217–222.
49. Maran E, Fernandez M, Barbieri P, Font G, Ruiz MJ. Effects of four carbamate compounds on antioxidant parameters. *Ecotoxicol Environ Saf* 2009; 72(3):922–930. doi: 10.1016/j.ecoenv.2008.01.018.
50. Rai DK, Rai PK, Rizvi SI, Watal G, Sharma B. Carbofuran-induced toxicity in rats: protective role of vitamin C. *Exp Toxicol Pathol* 2009; 61(6):531–535. doi: 10.1016/j.etp.2008.11.003.
51. Ellenhorn MJ, Schonwald S, Ordog G, Wasserberger J. *Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning*. Williams & Wilkins, Baltimore, Maryland, USA. 1997. pp. 221–5.
52. Toygar M, Aydin I, Agilli M, Aydin FN, Oztosun M, Gul H, et al. The relation between oxidative stress, inflammation, and neopterin in the paraquat-induced lung toxicity. *Hum Exp Toxicol* 2015; 34(2):198–204. doi: 10.1177/0960327114533808.
53. Jones GM, Vale JA. Mechanisms of toxicity, clinical features, and management of diquat poisoning: a review. *J Toxicol Clin Toxicol* 2000; 38(2):123–128.
54. He X, Wang L, Szklarz G, Bi Y, Ma Q. Resveratrol inhibits paraquat-induced oxidative stress and fibrogenic response by activating the nuclear factor erythroid 2-related factor 2 pathway. *J Pharmacol Exp Ther* 2012; 342(1):81–90. doi: 10.1124/jpet.112.194142.
55. Rodriguez-Rocha H, Garcia-Garcia A, Pickett C, Li S, Jones J, Chen H, et al. Compartmentalized oxidative stress in dopaminergic cell death induced by pesticides and complex I inhibitors: distinct roles of superoxide anion and superoxide dismutases. *Free Radic Biol Med* 2013; 61:370–383. doi: 10.1016/j.freeradbiomed.2013.04.021.
56. de Liz Oliveira Cavalli VL, Cattani D, Heinz Rieg CE, Pierozan P, Zanatta L, Benedetti Parisotto E, et al. Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells. *Free Radic Biol Med* 2013; 65:335–346. doi: 10.1016/j.freeradbiomed.2013.06.043.
57. Bukowska B, Rychlik B, Krokosz A, Michalowicz J. Phenoxyherbicides induce production of free radicals in human erythrocytes: oxidation of dichlorodihydrofluorescine and dihydrorhodamine 123 by 2,4-D-Na and MCPA-Na. *Food Chem Toxicol* 2008; 46(1):359–367. doi: 10.1016/j.fct.2007.08.011.
58. Drechsel DA, Patel M. Role of reactive oxygen species in the neurotoxicity of environmental agents implicated in Parkinson's disease. *Free Radic Biol Med* 2008; 44(11):1873–1886. doi: 10.1016/j.freeradbiomed.2008.02.008.
59. Hoogenraad TU. Dithiocarbamates and Parkinson's disease. *Lancet* 1988; 1(8588):767.
60. Meco G, Bonifati V, Vanacore N, Fabrizio E. Parkinsonism after chronic exposure to the fungicide maneb (manganese ethylene-bis-dithiocarbamate). *Scand J Work Environ Health* 1994; 20(4):301–305.
61. Domico LM, Zeevalk GD, Bernard LP, Cooper KR. Acute neurotoxic effects of mancozeb and maneb in mesencephalic neuronal cultures are associated with mitochondrial dysfunction. *Neurotoxicology* 2006; 27(5):816–825. doi: 10.1016/j.neuro.2006.07.009.
62. Regueiro J, Olguin N, Simal-Gandara J, Sunol C. Toxicity evaluation of new agricultural fungicides in primary cultured cortical neurons. *Environ Res* 2015; 140:37–44. doi: 10.1016/j.envres.2015.03.013.
63. Coleman MD, O'Neil JD, Woehrling EK, Ndunge OB, Hill EJ, Menache A, et al. A preliminary investigation into the impact of a pesticide combination on human neuronal and glial cell lines in vitro. *PLoS One* 2012;

7(8):e42768. doi: 10.1371/journal.pone.0042768.
64. Weidinger A, Kozlov AV. Biological activities of reactive oxygen and nitrogen species: oxidative stress versus signal transduction. *Biomolecules* 2015; 5(2):472–484. doi: 10.3390/biom5020472.
65. Purdom-Dickinson SE, Sheveleva EV, Sun H, Chen QM. Translational control of nrf2 protein in activation of antioxidant response by oxidants. *Mol Pharmacol* 2007; 72(4):1074–1081. doi: 10.1124/mol.107.035360.
66. Kobayashi M, Yamamoto M. Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. *Antioxid Redox Signal* 2005; 7(3–4):385–394. doi: 10.1089/ars.2005.7.385.
67. Cig B, Naziroglu M. Investigation of the effects of distance from sources on apoptosis, oxidative stress and cytosolic calcium accumulation via TRPV1 channels induced by mobile phones and Wi-Fi in breast cancer cells. *Biochim Biophys Acta* 2015; 1848(10 Pt B):2756–2765. doi: 10.1016/j.bbamem.2015.02.013.
68. Carloni M, Nasuti C, Fedeli D, Montani M, Vadhana MS, Amici A, et al. Early life permethrin exposure induces long-term brain changes in Nurr1, NF-kB and Nrf-2. *Brain Res* 2013; 1515:19–28. doi: 10.1016/j.brainres.2013.03.048.
69. Park JH, Park YS, Lee JB, Park KH, Paik MK, Jeong M, et al. Meloxicam inhibits fipronil-induced apoptosis via modulation of the oxidative stress and inflammatory response in SH-SY5Y cells. *J Appl Toxicol* 2016; 36(1):10–23. doi: 10.1002/jat.3136.
70. Lee JE, Kang JS, Ki YW, Lee SH, Lee SJ, Lee KS, et al. Akt/GSK3beta signaling is involved in fipronil-induced apoptotic cell death of human neuroblastoma SH-SY5Y cells. *Toxicol Lett* 2011; 202(2):133–141. doi: 10.1016/j.toxlet.2011.01.030.
71. Abu-Qare AW, Abou-Donia MB. DEET (N,N-diethyl-m-toluamide) alone and in combination with permethrin increased urinary excretion of 6beta-hydroxycortisol in rats, a marker of hepatic CYP3A induction. *J Toxicol Environ Health A* 2001; 64(5):373–384. doi: 10.1080/152873901753170722.
72. de Oliveira DC, Hastreiter AA, Mello AS, de Oliveira Beltran JS, Oliveira Santos EW, Borelli P, et al. The effects of protein malnutrition on the TNF-RI and NF-kappaB expression via the TNF-alpha signaling pathway. *Cytokine* 2014; 69(2):218–225. doi: 10.1016/j.cyto.2014.06.004.
73. Kantari C, Walczak H. Caspase-8 and bid: caught in the act between death receptors and mitochondria. *Biochim Biophys Acta* 2011; 1813(4):558–563. doi: 10.1016/j.bbamcr.2011.01.026.
74. Fedeli D, Montani M, Carloni M, Nasuti C, Amici A, Gabbianelli R. Leukocyte Nurr1 as peripheral biomarker of early-life environmental exposure to permethrin insecticide. *Biomarkers* 2012; 17(7):604–609. doi: 10.3109/1354750X.2012.706641.
75. Montarolo F, Raffaele C, Perga S, Martire S, Finardi A, Furlan R, et al. Effects of isoxazolo-pyridinone 7e, a potent activator of the Nurr1 signaling pathway, on experimental autoimmune encephalomyelitis in mice. *PLoS One* 2014; 9(9):e108791. doi: 10.1371/journal.pone.0108791.
76. Blanco-Ayala T, Anderica-Romero AC, Pedraza-Chaverri J. New insights into antioxidant strategies against paraquat toxicity. *Free Radic Res* 2014; 48(6):623–640. doi: 10.3109/10715762.2014.899694.