Marjanović Čermak AM, et al. Redox imbalance caused by pesticides
Arh Hig Rada Toksikol 2018;69:126-134

126

Redox imbalance caused by pesticides: a review of OPENTOX-related research

Ana Marija Marjanović Čermak, Ivan Pavičić, and Davor Želježić

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Pesticides are a highly diverse group of compounds and the most important chemical stressors in the environment. Mechanisms that could explain pesticide toxicity are constantly being studied and their interactions at the cellular level are often observed in well-controlled *in vitro* studies. Several pesticide groups have been found to impair the redox balance in the cell, but the mechanisms leading to oxidative stress for certain pesticides are only partly understood. As our scientific project "Organic pollutants in environment – markers and biomarkers of toxicity (OPENTOX)" is dedicated to studying toxic effects of selected insecticides and herbicides, this review is focused on reporting the knowledge regarding oxidative stress-related phenomena at the cellular level. We wanted to single out the most important facts relevant to the evaluation of our own findings from studies conducted on *in vitro* cell models.

KEY WORDS: *antioxidants; apoptosis; glyphosate;* in vitro*; neonicotinoids; organophosphates; oxidative stress; pyrethroids; reactive oxygen species*

Over the years, population growth and changes in food consumption patterns have challenged agricultural production to meet the demand for food and quality standards. This has led to an increased use of pesticides.

Pesticides are defined as "any substance or mixture of substances of chemicals or biological ingredients intended for repelling, destroying or controlling any pest, or regulating plant growth" (1). There is a wide range of pesticide types, including insecticides, herbicides, rodenticides, and fungicides. Because of their recognised potential to adversely affect untargeted biological systems, they have been studied extensively for their toxicity and associated risks (2, 3).

For the last two decades, toxicological research has been focused on oxidative stress as a possible mechanism of pesticide toxicity, but the precise mechanisms by which pesticides affect human metabolism at the cellular level are still unclear. Their toxic effects usually depend on the chemical structure of a pesticide, dose received, and time of exposure (4). Many of them are believed to be mediated by the regulation of apoptosis and redox signalling (5). Pesticides have been shown to induce apoptosis by activating signalling pathways mediated by mitochondria and DNA damage as well as through activation of death receptors (6).

As our scientific project "Organic pollutants in environment – markers and biomarkers of toxicity (OPENTOX)", financed by the Croatian Science Foundation

---
Correspondence to: Ana Marija Marjanović Čermak, PhD, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, Zagreb, Croatia, E-mail: *amarjanovic@imi.hr*

(HrZZ), is dedicated to studying the toxic effects of two major pesticide classes with three subgroups each: (A) insecticides (organophosphates, neonicotinoids, and pyrethroids) and (B) herbicides (triazines, organophosphates, allelopathic compounds) (7), this review is focused on reporting the knowledge regarding oxidative stress-related phenomena at the cellular level. Although the amount of information on this issue is remarkable, we selected only those groups (or single compounds) which are covered by our project, as we wanted to single out the most important facts relevant to the evaluation of our own findings from studies conducted on *in vitro* cell models.

REACTIVE OXYGEN SPECIES AND OXIDATIVE STRESS

Reactive oxygen species (ROS) are products of normal cell metabolism and metabolism of cells affected by xenobiotics. Their effects in the cell can be beneficial or harmful, depending on their concentration (8). Many of the ROS are free radicals, such as hydroxyl, peroxyl, superoxide, or nitric oxide, with one or more unpaired electrons, which makes them unstable and reactive. Seeking stability, radicals attack nearby molecules to obtain another electron, but in the process damage the structure and function of the attacked molecules (9). ROS interact with receptors, second messengers, and transcription factors that alter gene expression and influence cell growth and survival.

ROS attack three targets in the cell: proteins, DNA, and membrane lipids (10). The brain is particularly vulnerable to oxidative injury due to its high oxygen consumption, low

127

Marjanović Čermak AM, et al. Redox imbalance caused by pesticides
Arh Hig Rada Toksikol 2018;69:126-134

antioxidant defence, and high content of polyunsaturated fatty acids, which are easily oxidised. Lipid peroxidation can alter membrane fluidity, inactivate membrane-bound receptors or enzymes, and impair normal cell function and membrane permeability (9). Dopaminergic cells are particularly sensitive to ROS, because dopamine metabolism creates hydrogen peroxide and superoxide radicals (11). Dopaminergic neurons mainly develop after birth, which makes the developing nervous system highly sensitive to pesticides (12, 13).

Under normal conditions the levels of ROS and antioxidants is balanced. Their imbalance, manifested in an excess of ROS or lack of antioxidants or both, is what causes oxidative stress (8, 14).

INSECTICIDES

Organophosphates

Organophosphorus pesticides (OP) are widely used in agriculture due to their high effectiveness and a relatively short half-life in the environment. However, they are generally more toxic to vertebrates than other classes of insecticides (15), as they inhibit the activity of acetylcholinesterase (AChE), which causes accumulation of acetylcholine (ACh) at the neuronal synapses and neuromuscular junctions and results in convulsions, paralysis, and death (16). OPs also affect DNA and RNA synthesis, signal transduction pathways, and expression of different transcription factors, and cause oxidative stress, as described above (17-20). One such OP that causes oxidative stress even at low concentrations is chlorpyrifos (21). It also disturbs neurotransmission (22), inhibits the replication of cells in the nervous system (23), and disrupts neuronal differentiation (24). A study on PC12 cells, which are used as a standard model for neural cell differentiation, showed immediate increase in ROS after chlorpyrifos exposure (25). Because a developing nervous system is less capable of scavenging free radicals than adult, oxidative stress is a likely mechanism by which chlorpyrifos damages immature brain. Lee et al. (26) reported that chlorpyrifos inhibited mitochondrial activity in PC12 cells, which led to excessive ROS formation, cytochrome c release from mitochondria, and activation of apoptotic cell death. In this process, mitogen-activated protein kinase (MAPK) signalling played a crucial role in dopaminergic cell death. Moreover, exposure to chlorpyrifos altered the expression of proteins involved in antioxidant defence.

In SH-SY5Y cells paraoxon, parathion, phenyl saligenin phosphate, tri-ortho-tolyl phosphate, and triphenyl phosphite induced similar forms of time-dependent cell death. Their cytotoxicity manifested itself in nuclear condensation, budding, fragmentation, and caspase-3 activation (27).

A similar effect was seen after chlorpyrifos exposure (28). In a study of Raszewski et al. (5) chlorpyrifos induced death in SH-SY5Y cells by down-regulating anti-apoptotic Bcl-2 and Bcl-xL and increasing caspase-3 activity. These changes point to mitochondrial dysfunction and consequent apoptosis. Mitochondrial membrane potential is commonly used to evaluate mitochondrial function as an indicator of cell health. The stability of mitochondrial membrane preserves the dynamic equilibrium of intracellular free calcium concentrations, and their decline may also lead to apoptosis (29). A recent study (30) showed higher free Ca^{2+} concentrations, higher plasma membrane potential, and lower mitochondrial transmembrane potential in HepG2 cells after a 24-hour exposure to chlorpyrifos. In another *in vitro* study (31) chlorpyrifos-ethyl induced lipid peroxidation and disturbed the activity of antioxidant enzymes in erythrocytes, which suggests an involvement of ROS in the toxic effects of OP pesticides.

Oxidative stress was also observed in human erythrocytes after exposure to malathion *in vitro* (32). In this study the increased levels of malondialdehyde (MDA) pointed to lipid peroxidation. It was reduced by pretreatment with vitamins C and E but only where malathion levels were low (25 μmol L^{-1} and 75 μmol L^{-1}).

Neonicotinoids

Neonicotinoid insecticides are neurotoxicants that act as nicotinic acetylcholine receptor (nAChR) agonists in insects and mammals. Compared to OPs, they are considered less toxic to vertebrates due to preference for insect receptors (10). Today, they make one third of the global insecticide market. Among them imidacloprid was the world's top selling insecticide in 2010 (33). In spite of the original belief that neonicotinoids are only mildly toxic to mammals, there is increasing evidence of a variety of toxic effects on animals and humans (34-37). *In vitro* studies reported that imidacloprid disrupted the glutathione redox cycle by affecting its components glutathione reductase (GR), glutathione peroxidase (GPX), and glutathione-S-transferase (GST) in Chinese hamster ovary cells (CHO_{K1}) (33) and to activate the ERK cascade via nAChRs and intracellular calcium mobilisation in mouse N1E-115 neuroblastoma cells (38), which may affect the functioning of the neurons. The effects of imidacloprid were also studied in human lymphocytes and HepG2 cells after four- and 24-hour exposure to concentrations corresponding to the acceptable daily intake (ADI), residential exposure level (REL), and occupational exposure level (OEL) (39). The results showed that the applied imidacloprid concentrations did not trigger significant lipid peroxidation nor did they affect the total antioxidative capacity of lymphocytes or HepG2 cells (39).

On the other hand, a number of *in vivo* animal models have reported oxidative stress as neonicotinoids' mechanism of action (40-43). So far, it is still not clear whether

Marjanović Čermak AM, et al. Redox imbalance caused by pesticides
Arh Hig Rada Toksikol 2018;69:126-134

128

Table 1 *An overview of* in vitro *studies of oxidative stress induced by pesticides*

Pesticide type	Cell type / cell line	Antioxidant status and oxidative stress	Ref.
Chlorpyrifos	human neuroblastoma (SH-SY5Y)	Increased ROS level and concentration of MDA Increased level of cytosolic cytochrome c Increased level of cleaved caspase-9, caspase-3, and PARP MAPK activation	(21)
Chlorpyrifos	rat pheochromocytoma cell line (PC12)	Increased ROS production	(25)
Chlorpyrifos	rat pheochromocytoma cell line (PC12)	Increased level of cytosolic cytochrome c and increased level of cleaved caspase-9, caspase-3, and PARP Increased ROS generation, Increased concentration of MDA Significant decrease of mitochondrial complex I activity Altered expression of antioxidant enzymes (CuZnSOD, MnSOD) MAPK activation, apoptosis	(26)
Chlorpyrifos Chlorpyrifos+ Cypermethrin	human neuroblastoma (SH-SY5Y)	Down-regulation of *Bcl-2* and *Bcl-xL* Increased caspase 3 activation	(5)
Chlorpyrifos	hepatocellular carcinoma (HepG2)	Increased concentration of free intracellular Ca^{2+} and plasma membrane potential Decrease in mitochondrial transmembrane potential Slight apoptosis	(30)
Chlorpyrifos-ethyl	erythrocytes	Increased concentrations of MDA and GPX activity Decreased activity of SOD and CAT	(31)
Malathion	erythrocytes	Increased level of MDA and decreased SOD, CAT and GPX activity Vitamins C and E prevented induced changes at low concentrations of malathion	(32)
α-cypermethrin Chlorpyrifos Imidacloprid	hepatocellular carcinoma (HepG2) human lymphocytes	Oxidative stress biomarkers were not significantly altered	(39)
Abamectin Chlorfenapyr Imidacloprid	Chinese hamster ovary (CHO_{K1})	Significant inhibition of GST and GPX activity	(33)
Desnitro-imidacloprid (imidacloprid metabolite)	mouse neuroblastoma (N1E-115)	Activated MAPK/ERK signalling cascade at low concentrations Calcium mobilization	(38)
Fenvalerate and its metabolite p-chlorophenyl isovaleric acid (p-CPIA)	erythrocytes	Increased TBARS levels Decreased activity of antioxidant enzymes CAT, SOD, GR and GST	(51)
Bifenthrin	erythrocytes	Enhanced lipid peroxidation Decreased enzyme activity of CAT and SOD	(52)
Atrazine	rat pheochromocytoma cell line (PC12)	Decreased CAT activity and GSH levels Increased ROS level, lipid peroxidation, activity of GPX and GR Induced apoptosis Up-regulation of mRNA expression of *Bax*, p53, caspase-3, caspase-9 and down-regulation of *Bcl-2*	(66)

129

Marjanović Čermak AM, et al. Redox imbalance caused by pesticides
Arh Hig Rada Toksikol 2018;69:126-134

Pesticide type	Cell type / cell line	Antioxidant status and oxidative stress	Ref.
Terbuthylazine	peripheral blood isolated lymphocytes and HepG2 cells	ROS level increased (plasma, lymphocytes) ROS level no effect (HepG2) SOD and GPX decreased (whole blood, erythrocytes) SOD and GPX increased (HepG2, lymphocytes) Increased lipid peroxidation (plasma)	(67)
Glyphosate	human keratinocyte cell line (HaCaT)	Increased ROS production, cytoskeleton disorganization and chromatin condensation Induction of apoptosis	(76)
Glyphosate	mouse fibroblast cells (3T3-L1)	Increased activity of caspase-3 Induction of apoptosis	(78)
Glyphosate	hepatocellular carcinoma (HepG2)	Decreased lipid peroxidation, TAC and GPX No significant difference in the level of ROS and GSH	(75)

Bax – *Bcl-2-associated X gene;* Bcl2 – *B-cell lymphoma 2;* Bcl-xL – *B-cell lymphoma-extra large; CAT – catalase; ERK – extracellular-signal-regulated kinase; GPX – glutathione peroxidase; GR – glutathione reductase; GSH – glutathione; GST – glutathione-S-transferase; MAPK – mitogen-activated protein kinase; MDA – malondialdehyde; mRNA – messenger RNA; PARP – poly (ADP-ribose) polymerase; ROS – reactive oxygen species; SOD – superoxide dismutase; TAC – total antioxidant capacity; TBARS – thiobarbituric acid reactive substances*

oxidative stress is a secondary effect or has nothing to do with the nAChR agonists. As the application of neonicotinoids continues to grow, further research of their toxicity to vertebrates and invertebrates is absolutely necessary.

Pyrethroids

Pyrethroids are synthetic analogues and derivatives of natural insecticides pyrethrins obtained from the flowers of pyrethrum (*Chrysanthemum cinerariaefolium*) (44). Pyrethroids are extensively used in agriculture and indoors. They were meant to replace restricted or banned organophosphates but were also found to cause adverse effects (45). Because of their lipophilicity, they tend to act on biological membranes. They react with voltage-gated sodium nerve channels and prolong the time during which the channels are open (46). Because the structure and function of voltage-gated sodium channels are very similar between insects and mammals, mammals may also be affected by this mechanism of action.

It is likely, however, that voltage-gated sodium channels are not the only pyrethroid target (47). Pyrethroids were found to indirectly generate superoxide radicals, reactive nitrogen species such as peroxynitrite, nitric oxide, and hydroxyl radicals, causing damage consistent with oxidative stress (48, 49), whose likely target are erythrocytes because of high content of polyunsaturated fatty acids in cell membranes and elevated concentrations of oxygen and haemoglobin (50). In a study of Prasanthi et al. (51) another common insecticide, fenvalerate, induced oxidative stress in erythrocytes *in vitro* through lipid peroxidation and inhibition of antioxidant enzymes. Similar results in erythrocytes were observed after bifenthrin (52) and lambda-cyhalothrin exposure (53).

Cypermethrin is a class II synthetic pyrethroid pesticide that crosses the blood-brain barrier, affects the central nervous system, and impairs motor function (54). Although considered the safest pesticide, studies have shown its connection to developmental neurotoxicity, oxidative stress, and apoptosis (55-57). Cypermethrin seems to generate reactive oxygen and nitrogen species and reduce the antioxidant levels through its metabolites mediated by the cytochrome P450 2E1 (54).

HERBICIDES

Triazine herbicides

Triazine herbicides inhibit electron transport in photosynthesis and have been used as selective herbicides in agriculture for more than 50 years. They include asymmetrical triazines or triazinones (metribuzin) and symmetrical triazines. The major commercial symmetrical triazines are further divided into chloro-s-triazines (atrazine, propazine, terbutylazine), thiomethyl-s-triazines (ametryn, terbutryn), and methoxy-s-triazine (prometon) (58). Recent years saw a growing concern about the toxicity and environmental persistence and mobility of triazines and their metabolites (59). Atrazine has been used extensively, mainly due to its low cost and ease of application and is the most common contaminant of groundwater and surface water. Because of its persistence in the environment and toxicity for wildlife and possible effects on human health, it was banned in the EU in 2004 (60). Even so, it remains a significant environmental and biological hazard (61). The connection between atrazine and oxidative stress was observed in different *in vivo* and *in vitro* studies. Song et

Marjanović Čermak AM, et al. Redox imbalance caused by pesticides
Arh Hig Rada Toksikol 2018;69:126-134

130

al. (62) detected genotoxic effects of atrazine trough the formation of ROS, while Zhang et al. (63) reported enhanced lipid peroxidation and activity of antioxidant enzymes in male Wistar rats exposed to atrazine. Oxidative stress induced by atrazine was also detected in two bacterial strains (64) and adult female zebrafish (65). In addition, atrazine was found to induce apoptosis in PC12 cells by altering the expression of p53, caspase-3, and caspase-9 (66) and to cause apoptosis-related neurodegenerative damage in the nerve cells (61).

Unlike for atrazine, *in vitro* research has produced very limited knowledge about the oxidative stress caused by other triazine herbicides. We investigated the *in vitro* effects of a four-hour exposure to terbuthylazine concentrations of 8.00, 0.80, and 0.58 ng mL^{-1} in whole peripheral blood, isolated lymphocytes, and HepG2 cells (67), which is comparable with current reference values set by the European Commission in 2011 (68). ROS levels in plasma were significantly increased by all terbuthylazine concentrations, and in lymphocytes by the concentrations of 0.80 and 0.58 ng mL^{-1}, while no effect was observed in HepG2 cells. The activities of GPX in whole blood and of superoxide dismutase (SOD) in erythrocytes dropped, while in HepG2 cells and lymphocytes they rose possibly in response to oxidative/antioxidative disequilibrium. Significantly increased lipid peroxidation was only observed in plasma at the highest tested concentration (67).

In vivo studies showed that exposure to metribuzin was associated with lipid peroxidation and impaired antioxidant activity in crayfish (69) and oxidative stress in goldfish (70). Common carps in their embryo-larval stages were also affected by terbuthylazine and metribuzin through oxidative stress (71).

Glyphosate

Glyphosate is a non-selective, broad-spectrum, systemic organophosphorus herbicide for all plant types. It interferes with the production of the aromatic amino acids phenylalanine, tyrosine, and tryptophan, which are essential for plant growth (72). Since the pathway operates only in plants and microorganisms, glyphosate had long been considered safe for humans (73). Recently, however, the WHO and IARC changed its classification to probably carcinogenic (group 2A) to acknowledge doubts about its safety at low doses (74).

The effects of low doses of glyphosate on HepG2 cells were also tested in our research study (75). The results showed no increase in the ROS levels after four- and 24-hour exposure, indicating that ROS was efficiently removed by antioxidant defences. This was also confirmed by lower total antioxidant capacity (TAC). GPX activity dropped significantly after the four-hour treatment with the ADI concentrations while the OEL concentrations lowered it only after the 24-hour treatment.

Glyphosate was also found to adversely affect HaCaT cell adhesion potential, trigger hydrogen peroxide production and chromatin condensation, disrupt the cytoskeleton, and eventually induce apoptosis (76). The induction of apoptosis was confirmed in a variety of cell cultures (77-79).

Research has shown that ingredients added to commercial glyphosate formulations may influence the cytotoxicity of glyphosate and other herbicides (80-82). Coalova et al. (83), for example, showed that the addition of an adjuvant (alkyl-aryl-polyglycol ether) to glyphosate formulation increased its toxicity to HepG2 cells. The adjuvant increased ROS production, catalase activity, and glutathione concentrations. Moreover, this glyphosate formulation activated caspase 3/7 and the apoptosis pathway. An earlier study (84) has shown that glyphosate-based formulations can be responsible for oxidative damage to human epidermal cells (HaCaT).

4-hydroxyphenylpyruvate dioxygenase inhibitors

Recent bans of various agrochemicals in many European countries have created a demand for new effective compounds. Their development is mostly focused on targeting enzymes. One such enzyme is 4-hydroxyphenylpyruvate dioxygenase (HPPD), as it catalyses the initial steps in the tyrosine degradation pathway (85). In plants, this enzyme regulates growth, and its inhibition impairs photosynthesis, followed by leaf bleaching (86). In animals, it regulates blood tyrosine levels, and its inhibition results in blood tyrosine accumulation (87).

There are three main classes of commercial HPPD-inhibiting herbicides that specifically target a variety of broadleaf weeds without affecting the crops, whose application rate and toxicity is low, and which can be used for pre- and post-emergence treatment (88). These are izoxazoles, pyrazoles, and triketones (89). So far, the triketone herbicide family has not been proven unsafe for the environment and human health and is considered "eco-friendly" due to its "natural" origin and rapid degradation. Yet even "natural" products can have toxic effects on the environment. Beside leaf bleaching, these herbicides can trigger oxidative stress that disrupts cell metabolism. A toxicity study of sulcotrione (90) showed up-regulation of the genes inducing ROS and antioxidant enzymes in the fava bean cells (*Vicia faba*), followed by increased lipid peroxidation. Some pathogens, in turn, such as *E. coli* DH5-α (91) and *Pantoea ananatis* (92), showed resistance to oxidative stress caused by mesotrione, while human liver cancer HepG2 cells were only mildly affected by tembotrione (93).

Because these chemicals are relatively new, knowledge about their *in vitro* or *in vivo* effects is still very modest, and their extensive use and the formation of many

131

Marjanović Čermak AM, et al. Redox imbalance caused by pesticides
Arh Hig Rada Toksikol 2018;69:126-134

degradation products call for intensive research and real risk assessment.

Conflicts of interest

None to declare.

Acknowledgement

REFERENCES

1. Food and Agriculture Organization of the United Nations (FAO). The International Code of Conduct on Pesticide Management. Rome: FAO/WHO; 2014.

2. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. Pesticides and oxidative stress: a review. Med Sci Monit 2004;10:RA141-7. PMID: 15173684

3. Lee BW, Oh SH, Chung JH, Moon CK, Lee BH. *N*-Nitroso metabolite of carbofuran induces apoptosis in CHL cells by cytochrome *c*-mediated activation of caspases. Toxicology 2004;201:51-8. doi: 10.1016/j.tox.2004.03.022

4. Jabłońska-Trypuć A. Pesticides as inducers of oxidative stress. ROS 2017;3:96-110. doi: 10.20455/ros.2017.823

5. Raszewski G, Lemieszek MK, Łukawski K, Juszczak M, Rzeski W. Chlorpyrifos and cypermethrin induce apoptosis in human neuroblastoma cell line SH-SY5Y. Basic Clin Pharmacol Toxicol 2015;116:158-67. doi: 10.1111/bcpt.12285

6. Green DR, Reed JC. Mitochondria and apoptosis. Science 1998;281:1309-12. doi: 10.1126/science.281.5381.1309

7. Organic Pollutants in Environment-Markers and Biomarkers of Toxicity (OPENTOX). [displayed 16 May 2018]. Available at http://opentox.imi.hr/.

8. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med 2010;48:749-62. doi: 10.1016/j.freeradbiomed.2009.12.022

9. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 4th ed. New York (NY): Oxford University Press Inc; 2007.

10. Wang X, Anadón A, Qinghua W, Qiao F, Ares I, Martínez-Larrañaga MR, Yuan Z, Martínez MA. Mechanism of neonicotinoid toxicity: impact on oxidative stress and metabolism. Annu Rev Pharmacol Toxicol 2018;58:471-507. doi: 10.1146/annurev-pharmtox-010617-052429

11. Bharath S, Hsu M, Kaur D, Rajagopalan S, Andersen JK. Glutathione, iron and Parkinson's disease. Biochem Pharmacol 2002;64:1037-48. PMID: 12213603

12. Bonneh-Barkay D, Langston WJ, Di Monte DA. Toxicity of redox cycling pesticides in primary mesencephalic cultures. Antioxid Redox Sign 2005;7:649-53. doi: 10.1089/ars.2005.7.649

13. Kitazawa M, Anantharam V, Kanthasamy AG. Dieldrin-induced oxidative stress and neurocheminal changes contribute to apoptotic cell death in dopaminergic cells. Free Radic Biol Med 2001;31:1473-85. PMID: 11728820

14. Waris G, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinog 2006;5:14. doi: 10.1186/1477-3163-5-14

15. Dikshith TSS, Diwan PV. Organophosphorus pesticides. In: Industrial Guide to Chemical and Drug Safety. New Jersey, NJ: John Wiley & Sons, Inc; 2003. p. 123-56.

16. Ware GW, Whitacre DM. The Pesticide Book. 6th ed. Willoughby (OH): MeisterPro Information Resources; 2004.

17. Garcia SJ, Seidler FJ, Crumpton TL, Slotkin TA. Does the developmental neurotoxicity of chlorpyrifos involve glial targets? Macromolecule synthesis, adenylyl cyclase signaling, nuclear transcription factors, and formation of reactive oxygen in C6 glioma cells. Brain Res 2001;891:54-68. doi: 10.1016/S0006-8993(00)03189-9

18. Crumpton TL, Seidler FJ, Slotkin TA. Developmental neurotoxicity of chlorpyrifos *in vivo* and *in vitro*: effects on nuclear transcription factors involved in cell replication and differentiation. Brain Res 2000;857:87-98. doi: 10.1016/S0006-8993(99)02357-4

19. Song X, Seidler FJ, Saleh JL, Zhang J, Padilla S, Slotkin TA. Cellular mechanisms for developmental toxicity of chlorpyrifos: targeting the adenyl cyclase signaling cascade. Toxicol Appl Pharm 1997;145:158-74. doi: 10.1006/taap.1997.8171

20. Schuh RA, Lein PJ, Beckles RA, Jett DA. Noncholinesterase mechanisms of chlorpyrifos neurotoxicity: altered phosphorylation of Ca^{2+}/cAMP response element binding protein in cultured neurons. Toxicol Appl Pharm 2002;182:176-85. doi: 10.1006/taap.2002.9445

21. Ki YW, Park JH, Lee JE, Shin IC, Koh HC. JNK and p38 MAPK regulate oxidative stress and the inflammatory response in chlorpyrifos-induced apoptosis. Toxicol Lett 2013;218:235-45. doi: 10.1016/j.toxlet.2013.02.003

22. Slotkin TA, Seidler FJ. Prenatal chlorpyrifos exposure elicits presynaptic serotonergic and dopaminergic hyperactivity at adolescence: critical periods for regional and sex-selective effects. Reprod Toxicol 2007;23:421-7. doi: 10.1016/j.reprotox.2006.07.010

23. Qiao D, Seidler FJ, Slotkin TA. Developmental neurotoxicity of chlorpyrifos modeled *in vitro*: comparative effects of metabolites and other cholinesterase inhibitors on DNA synthesis in PC-12 and C-6 glioma cells. Environ Health Perspect 2001;109:909-13. doi: 10.1289/ehp.01109909

24. Yang D, Howard A, Bruun D, Ajua-Alemanj M, Pickart C, Lein PJ. Chlorpyrifos and chlorpyrifos-oxon inhibit axonal growth by interfering with the morphogenic activity of acetylcholinesterase. Toxicol Appl Pharma 2008;228:32-41. doi: 10.1016/j.taap.2007.11.005

25. Crumpton TL, Seidler FJ, Slotkin TA. Is oxidative stress involved in the developmental neurotoxicity of chlorpyrifos? Dev Brain Res 2000;121:189-95. doi: 10.1016/S0165-3806(00)00045-6

26. Lee JE, Park JH, Shin IC, Koh HC. Reactive oxygen species regulated mitochondria-mediated apoptosis in PC12 cells exposed to chlorpyrifos. Toxicol Appl Pharma 2012;263:148-62. doi: 10.1016/j.taap.2012.06.005

27. Carlson K, Jortner BS, Ehrich M. Organophosphorus compound-induced apoptosis in SH-SY5Y human neuroblastoma cells. Toxicol Appl Pharm 2000;168:102-13. doi: 10.1006/taap.2000.8997

Marjanović Čermak AM, et al. Redox imbalance caused by pesticides
Arh Hig Rada Toksikol 2018;69:126-134

132

28. Park JH, Lee JE, Shin IC, Koh HC. Autophagy regulates chlorpyrifos-induced apoptosis in SH-SY5Y cells. Toxicol Appl Pharm 2013;268:55-67. doi: 10.1016/j.taap.2013.01.013

29. Sakamuru S, Attene-Ramos MS, Xia M. Mitochondrial membrane potential assay. Methods Mol Biol 2016;1473:17-22. doi: 10.1007/978-1-4939-6346-1_2

30. Zhou C, Li X. Cytotoxicity of chlorpyrifos to human liver hepatocellular carcinoma cells: effects on mitochondrial membrane potential and intracellular free Ca^{2+}. Toxin Reviews 2017. doi: 10.1080/15569543.2017.1386686

31. Gultekin F, Ozturk M, Akdogan M. The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (in vitro). Arch Toxicol 2000;74:533-8. doi: 10.1007/s002040000167

32. Durak D, Uzun FG, Kalender S, Ogutcu A, Uzunhisarcikli M, Kalender Y. Malathion-induced oxidative stress in human erythrocytes and the protective effect of vitamins C and E in vitro. Environ Toxicol 2009;24:235-42. doi: 10.1002/tox.20423

33. Al-Sarar AS, Abobakr Y, Bayoumi AE, Hussein HI. Cytotoxic and genotoxic effects of abamectin, chlorfenapyr, and imidacloprid on CHO_{K1} cells. Environ Sci Pollut R 2015;22:17041-52. doi: 10.1007/s11356-015-4927-3

34. Bal R, Türk G, Tuzcu M, Yilmaz O, Kuloglu T, Gundogdu R, Gür S, Agca A, Ulas M, Cambay Z, Tuzcu Z, Gencoglu H, Guvenc M, Ozsahin AD, Kocaman N, Aslan A, Etem E. Assessment of imidacloprid toxicity on reproductive organ system of adult male rats. J Environ Sci Health B 2012;47:434-44. doi: 10.1080/03601234.2012.663311

35. Lonare M, Kumar M, Raut S, Badgujar P, Doltade S, Telang A. Evaluation of imidacloprid-induced neurotoxicity in male rats: a protective effect of curcumin. Neurochem Int 2014;78:122-9. doi: 10.1016/j.neuint.2014.09.004

36. Kapoor U, Srivastava MK, Bhardwaj S, Srivastava LP. Effect of imidacloprid on antioxidant enzymes and lipid peroxidation in female rats to derive its No Observed Effect Level (NOEL). J Toxicol Sci 2010;35:577-81. doi: 10.2131/jts.35.577

37. Yardimci M, Sevgiler Y, Rencuzogullari E, Arslan M, Buyukleyla M, Yilmaz M. Sex-, tissue-, and exposure duration-dependent effects of imidacloprid modulated by piperonyl butoxide and menadione in rats. Part I: oxidative and neurotoxic potentials. Arh Hig Rada Toksikol 2014;65:387-98. doi: 10.2478/10004-1254-65-2014-2554

38. Tomizawa M, Casida JE. Desnitro-imidacloprid activates the extracellular signal-regulated kinase cascade via the nicotinic receptor and intracellular calcium mobilization in N1E-115 cells. Toxicol Appl Pharm 2002;184:180-6. doi: 10.1006/taap.2002.9503

39. Želježić D, Mladinić M, Žunec S, Lucić Vrdoljak A, Kašuba V, Tariba B, Živković T, Marjanović AM, Pavičić I, Milić M, Rozgaj R, Kopjar N. Cytotoxic, genotoxic and biochemical markers of insecticide toxicity evaluated in human peripheral blood lymphocytes and an HepG2 cell line. Food Chem Toxicol 2016;96:90-106. doi: 10.1016/j.fct.2016.07.036.

40. Annabi A, Dhouib IB, Lamine AJ, El Golli N, Gharbi N, El Fazâa S, Lasram MM. Recovery by N-acetylcysteine from subchronic exposure to Imidacloprid-induced hypothalamic-pituitary-adrenal (HPA) axis tissues injury in male rats. Toxicol Mech Methods 2015;25:524-31. doi: 10.3109/15376516.2015.1045663

41. Soujanya S, Lakshman M, Kumar AA, Reddy AG. Evaluation of the protective role of vitamin C in imidacloprid-induced hepatotoxicity in male Albino rats. J Nat Sci Biol Med 2013;4:63-7. doi: 10.4103/0976-9668.107262

42. Zhang JJ, Wang Y, Xiang HY, Li MX, Li WH, Ma KG, Wang XZ, Zhang JH. Oxidative stress: role in acetamiprid-induced impairment of the male mice reproductive system. Agr Sci China 2011;10:786-96. doi: 10.1016/S1671-2927(11)60063-1

43. Yan S, Wang J, Zhu L, Chen A, Wang J. Toxic effects of nitenpyram on antioxidant enzyme system and DNA in zebrafish (Danio rerio) livers. Ecotoxicol Environ Saf 2015;122:54-60. doi: 10.1016/j.ecoenv.2015.06.030

44. Kaneko H. Pyrethroid chemistry and metabolism. In: Krieger R, editor. Hayes' Handbook of Pesticide Toxicology. 3rd ed. New York (NY): Academic Press; 2010. p. 1635-63.

45. Saillenfait AM, Ndiaye D, Sabaté JP. Pyrethroids: exposure and health effects-an update. Int J Hyg Envir Heal 2015;218:281-92. doi: 10.1016/j.ijheh.2015.01.002

46. Soderlund DM, Bloomquist JR. Neurotoxic actions of pyrethroid insecticides. Annu Rev Entomol 1989;34:77-96. doi: 10.1146/annurev.en.34.010189.000453

47. Soderlund DM. Toxicology and mode of action of pyrethroid insecticides. In: Krieger R, editor. Hayes' Handbook of Pesticide Toxicology. 3rd ed. New York (NY): Academic Press; 2010. p. 1665-86.

48. World Health Organization (WHO). Cyhalothrin. Environmental Health Criteria, 99. Geneva: WHO; 1990.

49. 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;105:197-205. doi: 10.1016/S0378-4274(98)00399-3

50. Clemens MR, Waller HD. Lipid peroxidation in erythrocytes. Chem Phys Lipids 1987;45:251-68. doi: 10.1016/0009-3084(87)90068-5

51. Prasanthi K, Muralidhara Rajini PS. Morphological and biochemical perturbations in rat erythrocytes following in vitro exposure to Fenvalerate and its metabolite. Toxicol In Vitro 2005;19:449-56. doi: 10.1016/j.tiv.2004.12.003

52. Sadowska-Woda I, Popowicz D, Karowicz-Bilińska A. Bifenthrin-induced oxidative stress in human erythrocytes in vitro and protective effect of selected flavonols. Toxicol in Vitro 2010;24:460-4. doi: 10.1016/j.tiv.2009.09.024

53. El-Demerdash FM. Lambda-cyhalothrin-induced changes in oxidative stress biomarkers in rabbit erythrocytes and alleviation effect of some antioxidants. Toxicol in Vitro 2007;21:392-7. doi: 10.1016/j.tiv.2006.09.019

54. Singh AK, Tiwari MN, Prakash O, Singh MP. A current review of cypermethrin-induced neurotoxicity and nigrostriatal dopaminergic neuro-degeneration. Curr Neuropharmacol 2012;10:64-71. doi: 10.2174/157015912799362779

55. Igbedioh SO. Effects of agricultural pesticides on humans, animals, and higher plants in developing countries. Arch Environ Health 1991;46:218-24. doi: 10.1080/00039896.1991.9937452

56. Maurya SK, Rai A, Rai NK, Deshpande S, Jain R, Mudiam MK, Prabhakar YS, Bandyopadhyay S. Cypermethrin induces astrocyte apoptosis by the disruption of the autocrine/paracrine mode of epidermal growth factor receptor

133

Marjanović Čermak AM, et al. Redox imbalance caused by pesticides
Arh Hig Rada Toksikol 2018;69:126-134

signaling. Toxicol Sci 2012;125:473-87. doi: 10.1093/toxsci/kfr303

57. Wang XZ, Liu SS, Sun Y, Wu JY, Zhou YL, Zhang JH. Beta-cypermethrin impairs reproductive function in male mice by inducing oxidative stress. Theriogenology 2009;72:599-611. doi: 10.1016/j.theriogenology.2009.04.016

58. Stevens JT, Breckenridge CB, Simpkins J, Eldridge JC. Symmetrical and asymmetrical triazine herbicides. In: Krieger R, editor. Handbook of Pesticide Toxicology. Vol 2. San Diego (CA): Academic Press; 2001. p. 1511-9.

59. Klementova S, Keltnerova L. Triazine herbicides in the environment. In: Price A, Kelton J, Sarunaite L, editors. Herbicides, Physiology of Action, and Safety. InTech, 2015. doi: 10.5772/60858

60. 2004/248/EC: Commission Decision of 10 March 2004 concerning the non-inclusion of atrazine in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing this active substance [displayed 9 May 2018], Available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32004D0248

61. Song XY, Li JN, Wu YP, Zhang B, Li BX. Atrazine causes autophagy- and apoptosis-related neurodegenerative effects in dopaminergic neurons in the rat nigrostriatal dopaminergic system. Int J Mol Sci 2015;16:13490-506. doi: 10.3390/ijms160613490

62. Song Y, Zhu LS, Wang J, Wang JH, Liu W, Xie H. DNA damage and effects on antioxidative enzymes in earthworm (*Eisenia foetida*) induced by atrazine. Soil Biol Biochem 2009;41:905-9. doi: 10.1016/j.soilbio.2008.09.009

63. Singh M, Sandhir R, Kiran R. Effects on antioxidant status of liver following atrazine exposure and its attenuation by vitamin E. Exp Toxicol Pathol 2011;63:269-76. doi: 10.1016/j.etp.2010.01.005

64. Zhang Y, Meng D, Wang Z, Guo H, Wang Y. Oxidative stress response in two representative bacteria exposed to atrazine. FEMS Microbiol Lett 2012;334:95-101. doi: 10.1111/j.1574-6968.2012.02625.x

65. Jin YX, Zhang XX, Shu LJ, Chen LF, Sun LW, Qian HF, Liu WP, Fu ZW. Oxidative stress response and gene expression with atrazine exposure in adult female zebrafish (*Danio rerio*). Chemosphere 2010;78:846-52. doi: 10.1016/j.chemosphere.2009.11.044

66. Abarikwu SO, Farombi EO, Kashyap MP, Pant AB. Kolaviron protects apoptotic cell death in PC12 cells exposed to Atrazine. Free Radical Res 2011;45:1061-73. doi: 10.3109/10715762.2011.593177

67. Želježić D, Žunec S, Bjeliš M, Benković V, Mladinić M, Lovaković Tariba B, Pavičić I, Marjanović Čermak AM, Kašuba V, Milić M, Pizent A, Lucić Vrdoljak A, Kopjar N. Effects of the chloro-s-triazine herbicide terbuthylazine on DNA integrity in human and mouse cells. Environ Sci Pollut Res 2018 [Epub ahead of print]. doi: 10.1007/s11356-018-2046-7.

68. European Commission. Review report for the active substance terbuthylazine finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 17 June 2011 in view of the approval of terbuthylazine as active substances in accordance with Regulation (EC) No 1107/2009. [displayed 17 May 2018]. Available at http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.ViewReview&id=467.

69. Koutnik D, Stara A, Zuskova E, Kouba A, Velisek J. The effect of subchronic metribuzin exposure to signal crayfish (*Pacifastacus leniusculus* Dana 1852). Neuro Endocrinol Lett 2014;35(Suppl 2):51-6. PMID: 25638366

70. Husak VV, Mosiichuk NM, Maksymiv IV, Sluchyk IY, Storey JM, Storey KB, Lushchak VI. Histopathological and biochemical changes in goldfish kidney due to exposure to the herbicide Sencor may be related to induction of oxidative stress. Aquat Toxicol 2014;155:181-9. doi: 10.1016/j.aquatox.2014.06.020

71. Hostovsky M, Blahova J, Plhalova L, Stepanova S, Praskova E, Marsalek P, Svobodova Z. Oxidative stress parameters in early developmental stages of common carp (*Cyprinus carpio* L.) after subchronic exposure to terbuthylazine and metribuzin. Neuro Endocrinol Lett 2012;33(Suppl 3):124-9. PMID: 23353855

72. Herrmann KM, Weaver LM. The shikimate pathway. Annu Rev Plant Physiol Plant Mol Biol 1999;50:473–503. doi: 10.1146/annurev.arplant.50.1.473

73. de María N, Becerril JM, Garca-Plazaola JI, Hernandez AH, de Felipe MR, Fernández-Pascual M. New insights on glyphosate mode of action in nodular metabolism: role of shikimate accumulation. J Agr Food Chem 1996;54:2621-8. doi: 10.1021/jf058166c

74. International Agency for Research on Cancer (IARC). Some Organophosphate Insecticides and Herbicides. IARC Monographs Vol. 112. Lyon: IARC; 2015.

75. Kašuba V, Milić M, Rozgaj R, Kopjar N, Mladinić M, Žunec S, Lucić Vrdoljak A, Pavičić I, Marjanović Čermak AM, Pizent A, Tariba Lovaković B, Želježić D. Effects of low doses of glyphosate on DNA damage, cell proliferation and oxidative stress in the HepG2 cell line. Environ Sci Pollut Res 2017;24(23):19267-81. doi: 10.1007/s11356-017-9438-y

76. Elie-Caille C, Heu C, Guyon C, Nicod L. Morphological damages of a glyphosate-treated human keratinocyte cell line revealed by a micro- to nanoscale microscopic investigation. Cell Biol Toxicol 2010;26:331-9. doi: 10.1007/s10565-009-9146-6

77. Gui YX, Fan XN, Wang HM, Wang G, Chen SD. Glyphosate induced cell death through apoptotic and autophagic mechanisms. Neurotoxicol Teratol 2012;34:344-9. doi: 10.1016/j.ntt.2012.03.005

78. Martini CN, Gabrielli M, Vila MC. A commercial formulation of glyphosate inhibits proliferation and differentiation to adipocytes and induces apoptosis in 3T3-L1 fibroblasts. Toxicol In Vitro 2012;26:1007-13. doi: 10.1016/j.tiv.2012.04.017

79. Kim YH, Hong JR, Gil HW, Song HY, Hong SY. Mixtures of glyphosate and surfactant TN20 accelerate cell death via mitochondrial damage-induced apoptosis and necrosis. Toxicol In Vitro 2013;27:191-7. doi: 10.1016/j.tiv.2012.09.021

80. Li MH. Effects of nonionic and ionic surfactants on survival, oxidative stress, and cholinesterase activity of planarian. Chemosphere 2008;70:1796-803. doi: 10.1016/j.chemosphere.2007.08.032

81. Song HY, Kim YH, Seok SJ, Gil HW, Hong SY. *In vitro* cytotoxic effect of glyphosate mixture containing surfactants. J Korean Med Sci 2012;27:711-5. doi: 10.3346/jkms.2012.27.7.711

82. Song HY, Kim YH, Seok SJ, Gil HW, Yang JO, Lee EY, Hong SY. Cellular toxicity of surfactants used as herbicide

Marjanović Čermak AM, et al. Redox imbalance caused by pesticides
Arh Hig Rada Toksikol 2018;69:126-134

134

additives. J Korean Med Sci 2012;27:3-9. doi: 10.3346/jkms.2012.27.1.3

83. Coalova I, Ríos de Molina Mdel C, Chaufan G. Influence of the spray adjuvant on the toxicity effects of a glyphosate formulation. Toxicol In Vitro 2014;28:1306-11. doi: 10.1016/j.tiv.2014.06.014

84. Gehin A, Guillaume YC, Millet J, Guyon C, Nicod L. Vitamins C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach. Int J Pharmaceut 2005;288:219-26. doi: 10.1016/j.ijpharm.2004.09.024

85. Norris SR, Shen X. DellaPenna D. Complementation of the Arabidopsis *pds1* mutation with the gene encoding *p*-hydroxyphenylpyruvate dioxygenase. Plant Physiol 1998;117:1317-23. PMCID: PMC34895

86. Lee DL, Knudsen CG, Michaely WJ, Chin H-L, Nguyen NH, Carter CG, Cromartie TH, Lake BH, Shribbs JM, Fraser T. The structure-activity relationships of the triketone class of HPPD herbicides. Pestic Sci 1998;54:377-84. doi: 10.1002/(SICI)1096-9063(199812)54:4<377::AID-PS827>3.0.CO;2-0

87. United States Environmental Protection Agency (US EPA). Human-Health Risk Assessment for Proposed Uses on Field Corn, Sweet Corn and Popcorn [displayed 18 May 2018]. Available at https://www.fluoridealert.org/wp-content/pesticides/EPA-HQ-OPP-2006-0072-0005

88. Ndikuryayo F, Moosavi B, Yang WC, Yang GF. 4-Hydroxyphenylpyruvate dioxygenase inhibitors: from chemical biology to agrochemicals. J Agr Food Chem 2017;65:8523-37. doi: 10.1021/acs.jafc.7b03851

89. Ahrens H, Lange G, Müller T, Rosinger C, Willms L van Almsick A. 4-hydroxyphenylpyruvate dioxygenase inhibitors in combination with safeners: solutions for modern and sustainable agriculture. Angew Chem Int Edit 2013;52:9388-98. doi: 10.1002/anie.201302365

90. Sta C, Goujon E, Ferjani E, Ledoigt G. Toxicity of sulcotrione and grape marc on *Vicia faba* cells. J Agr Food Chem 2014;62:11777-85. doi: 10.1021/jf503323t

91. Olchanheski LR, Dourado MN, Beltrame FL, Zielinski AA, Demiate IM, Pileggi SA, Azevedo RA, Sadowsky MJ, Pileggi M. Mechanisms of tolerance and high degradation capacity of the herbicide mesotrione by *Escherichia coli* strain DH5-α. PLoS One 2014;9(6):e99960. doi: 10.1371/journal.pone.0099960

92. Prione LP, Olchanheski LR, Tullio LD, Santo BCE, Reche PM, Martins PF, Carvalho G, Demiate IM, Pileggi SAV, Dourado MN, Prestes RA, Sadowsky MJ, Azevedo RA, Pileggi M. GST activity and membrane lipid saturation prevents mesotrione-induced cellular damage in *Pantoea ananatis*. AMB Express 2016;6:70. doi: 10.1186/s13568-016-0240-x

93. Žunec S, Kašuba V, Pavičić I, Marjanović AM, Tariba B, Milić M, Kopjar N, Pizent A, Vrdoljak AL, Rozgaj R, Želježić D. Assessment of oxidative stress responses and the cytotoxic and genotoxic potential of the herbicide tembotrione in HepG2 cells. Food Chem Toxicol 2016;94:64-74. doi: 10.1016/j.fct.2016.05.019

Oksidacijsko-redukcijska neravnoteža uzrokovana pesticidima: pregled istraživanja vezanih uz OPENTOX

Pesticidi su raznovrsna skupina spojeva i glavni izvor stresa u okolišu. Mehanizmi kojima se nastoji objasniti toksičnost pesticida kontinuirano se istražuju, a njihove interakcije na staničnoj razini često se promatraju u sklopu kontroliranih *in vitro* istraživanja. Za nekoliko skupina pesticida utvrđeno je da narušavaju oksidacijsko-redukcijsku ravnotežu u stanici, a mehanizmi koji vode do nastanka oksidacijskoga stresa za pojedine su pesticide još uvijek nedovoljno poznati. Budući da je naš znanstveni projekt „Organska zagađivala u okolišu-markeri i biomarkeri toksičnosti (OPENTOX)" posvećen istraživanju toksičnih učinaka odabranih insekticida i herbicida, ovaj pregledni rad usmjeren je na prikaz spoznaja koje se odnose na promjene uzrokovane stresom na staničnoj razini. Željeli smo izdvojiti najvažnije činjenice koje su bitne za procjenu vlastitih rezultata istraživanja provedenih na *in vitro* staničnim modelima.

KLJUČNE RIJEČI: *antioksidansi; apoptoza; glifosat;* in vitro*; neonikotinoidi; organofosfati; oksidacijski stres; piretroidi; reaktivni kisikovi spojevi*