ВІСНИК ЛЬВІВ. УН-ТУ
Серія біологічна. 2010. Вип. 54. С. 3-14

VISNYK OF LVIV UNIV.
Biology series. 2010. Is. 54. P. 3-14

Огляд

UDK 612.017:632.95:611.81

BIOLOGICAL EFFECTS ASSESSMENT OF CHLORPYRIFOS AND SOME ASPECTS OF ITS NEUROTOXICITY

Y. Salyha

Institute of Animal Biology of the NAAS of Ukraine
38, Vasyl Stus St., Lviv 79034 , Ukraine
e-mail: yursalyha@yahoo.com

In the present study a general survey is made of chlorpyrifos - one of the most commonly used organophosphorus pesticides in the world. The review does not give an exhaustive treatment of the biochemistry of chlorpyrifos. This question has been discussed at length in many reviews and will be briefly dealt with here. An attempt was made to review the vast amount of information concerning mechanisms of chlorpyrifos neurotoxicity. It has long been known, that chlorpyrifos is acetylcholinesterase inhibitor and that high sensitivity of the cholinesterases inhibitors makes them highly toxic to the central nervous system. Chlorpyrifos continues to receive considerable research interest. In the past five years there have been some innovations in the question concerning mechanisms of chlorpyrifos neurotoxicity, specifically it was repeatedly demonstrated that chlorpyrifos toxicity is not limited to cholinesterase inhibition alone but can act by other mechanisms. Here we summarize the existent literature available on this subject.

Key words: brain, central nervous system, chlorpyrifos, cholinesterase, neurotoxicity, organophosphate insecticides, pesticides.

Chlorpyrifos (CPF) is one of the most commonly used organophosphorus pesticides (OP) for domestic, agricultural and industrial purposes. The insecticide CPF is an OP that has been on the world market since 1965 to control insects in agriculture, gardens, building construction, and households. Trade names of the CPF include Brodan, Detmol UA, Dowco 179, Dursban, Empire, Eradex, Lorsban, Paqeant, Piridane, Scout, Stipend and other. Currently, for example only in the United States of America, there are over 850 registered CPF products. Despite recent restrictions on home use in certain countries, it remains a popular pesticide throughout the world. CPF is authorized for use in about 100 countries worldwide, including Ukraine, U.S.A., Canada, the United Kingdom, Spain, France, Italy, Japan, Australia, New Zealand, and most other developed nations. More than 50 crops, many of which are dietary staples for entire nations, are protected from insect infestation with chlorpyrifos products. In 2000, the National Center for Food and Agriculture Policy, Washington DC, estimated that up to 3 million pounds (1,4 million kg) of chlorpyrifos was being used in the home-and-garden market each year [12, 28]. The United Kingdom Advisory Committee on Pesticides (ACP) have recommended that use of CPF in home and garden products be revoked and have also raised concerns about the safety levels for the pesticide in food. In 2002 the use of CPF was restricted to only agricultural applications, and all domestic use was to be completely phased out by 1 January 2005 [12]. In 2005, agricultural uses of chlorpyrifos received European Union approvals in the form of inclusion in Annex I of the European Commission's Plant Protection Products Directive 91/414, allowing EU member states to renew their registrations of chlorpyrifos products. CPF is one of the most commonly used OP in Ukraine [1]. CPF is effective in controlling cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants,

and lice. It is used as an insecticide on grain, cotton, field, fruit, nut and vegetable crops, and well as on lawns and ornamental plants. It is also registered for direct use on sheep and turkeys, for horse site treatment, dog kennels, domestic dwellings, farm buildings, storage bins, and commercial establishments. CPF acts on pests primarily as a contact poison, with some action as a stomach poison. It does not mix well with water, so it is usually mixed with oily liquids before it is applied to crops or animals. It may also be applied to crops in a capsule form.

Physicochemical properties. CPF (IUPAC: *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate; CAS: *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate); CAS number: 2921-88-2; chemical formula: $C_9H_{11}Cl_3NO_3PS$ (Fig. 1); molecular weight: 350,62) is a clear to white crystalline solid pesticide with a strong mercaptan odor. Water solubility of CPF is 2 mg/L (25°C), it is soluble in benzene, acetone, chloroform, carbon disulfide, diethyl ether, xylene, methylene chloride ethanol and methanol. Melting point of CPF is 41,5–44°C; vapor pressure: 2,5 mPa (25°C); partition coefficient: 4,6990; soil adsorption coefficient Koc: 6070; hydrolysis half-life: 72 days (pH 7); aqueous photolysis half-life: 29,6 days (pH 7); aerobic soil metabolism: 76,9 days; aerobic aquatic metabolism ($t_{1/2}$): 153,8 days; anaerobic aquatic metabolism ($t_{1/2}$): 81,5 days [6, 52]. CPF is available as granules, wet table powder, dustable powder, and emulsifiable concentrate [52].

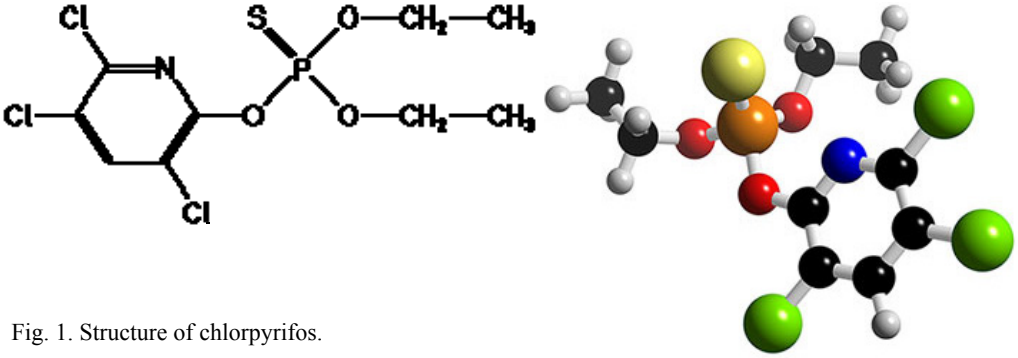

Fig. 1. Structure of chlorpyrifos.

Environmental fate. CPF is released into the environment primarily from its application as an insecticide. It is moderately persistent in soils. It is characterized by an average soil and sediment sorption coefficient (Koc) of 8498. The half-life of CPF in soil is usually between 60 and 120 days, but can range from 2 weeks to over 1 year, depending on the soil type, climate, and other conditions [6]. CPF adsorbs strongly to soil particles and it is not readily soluble in water. It is therefore immobile in soils and unlikely to leach or to contaminate groundwater [6]. It has been found to be relatively immobile vertically in soil and has not proved to be a groundwater contaminant. 3,5,6-trichloro-2-pyridinol (TCP), the principal metabolite of CPF, adsorbs weakly to soil particles and appears to be moderately mobile and persistent in soils. The concentration and persistence of CPF in water will vary depending on the type of formulation. For example, a large increase in CPF concentrations occurs when emulsifiable concentrations and wet table powders are released into water. As the pesticide adheres to sediments and suspended organic matter, concentrations rapidly decline. The increase in the concentration of insecticide is not as rapid for granules and controlled release formulations in the water, but the resulting concentration persists longer. Volatilization is probably the primary route of loss of CPF from water. Volatility half-lives of 3,5 and 20 days have been estimated for pond water [16]. The photolysis half-life of CPF is 3 to 4 weeks during midsummer in the U.S. Its change into other natural

forms is slow. Due to the nonpolar nature of CPF molecule, it possesses a low water solubility (< 2 ppm) and great tendency to partition from aqueous into organic phases in the environment (log P of 4,7–5,3). Research suggests that this insecticide is unstable in water, and the rate at which it is hydrolyzed increases with temperature, decreasing by 2,5- to 3-fold with each 10°C drop in temperature. The rate of hydrolysis is constant in acidic to neutral waters, but increases in alkaline waters. In water at pH 7,0 and 25°C, it had a half-life of 35 to 78 days [4, 39, 47].

CPF may oxidize in the environment to form chlorpyrifos-oxon. Studies have shown CPF-oxon can form up to nearly 100% of parent from drinking water treatment. Lesser amounts of oxon formation are expected in other media (soil, air, and surface water) however, insufficient data are currently available to quantify this amount. Chlorpyrifos-oxon, a minor degradation product of CPF, has been detected in environmental samples, including drinking water, surface water and precipitation. Toxicity data based on human health studies indicate that CPF-oxon is roughly 10 times more toxic than parent CPF [16, 30, 39].

Mark Corbin from the Environmental fate and effects division office of pesticide programs (Washington, USA) underline, that other pesticides may combine with CPF to produce synergistic, additive, and/or antagonistic toxic interactions. If CPF is present in the environment in combination with other chemicals, the toxicity of the mixture may be increased relative to the toxicity of each individual chemical, offset by other environmental factors, or even reduced by the presence of antagonistic contaminants if they were also present in the mixture. The variety of chemical interactions presented in the available data set suggest that the toxic effect of CPF, in combination with other pesticides used in the environment, can be a function of many factors including but not necessarily limited to (1) the exposed species, (2) the co-contaminants in the mixture, (3) the ratio of chlorpyrifos and co-contaminant concentrations, (4) differences in the pattern and duration of exposure among contaminants, and (5) the differential effects of other physical/chemical characteristics of the receiving waters (*e.g.* organic matter present in sediment and suspended water).

Metabolic pathway. It is very important to understand the mammalian and human metabolism of organophosphorus insecticides, CPF in particular (Fig. 2). Larry L. Needham from the Centers for Disease Control and Prevention (Atlanta, Georgia, USA) clearly described it at the work [30]. CPF is bioactivated by cytochrome P450-dependent desulfuration in the liver to chlorpyrifos-oxon. This oxon is rapidly hydrolyzed to its specific metabolite TCPy and to diethylphosphate (DEP) by microsomal esterases, which include PON1 and CPF oxonase, or by nonenzymatic hydrolysis. Alternatively, CPF is dearylated to form TCPy and diethylthiophosphate (DETP) by microsomal enzymes or by nonenzymatic hydrolysis. A complicating factor in interpreting CPF metabolite concentrations in urine is that these human metabolites and their environmental degradates are the same chemicals [5, 30, 35]. CPF is readily absorbed into the bloodstream through the gastrointestinal tract if it is ingested, through the lungs if it is inhaled, or through the skin if there is dermal exposure. In humans, CPF and its principal metabolites are eliminated rapidly [7]. After a single oral dose, the half-life of CPF in the blood appears to be about 1 day [48]. CPF is eliminated primarily through the kidneys [37]. CPF does not have a significant bioaccumulation potential. Following its oral intake by rats, 90% is removed in the urine and 10% is excreted in the feces [48]. It is detoxified quickly in rats, dogs, and other animals [48]. The major metabolite found in rat urine after a single oral dose is trichloropyridinol (TCP). TCP does not inhibit cholinesterase and it is not mutagenic.

General toxicological effects. The pesticide CPF is used to kill a broad range of insects and mites but it is toxic to most living organisms and humans. The available mammalian acute

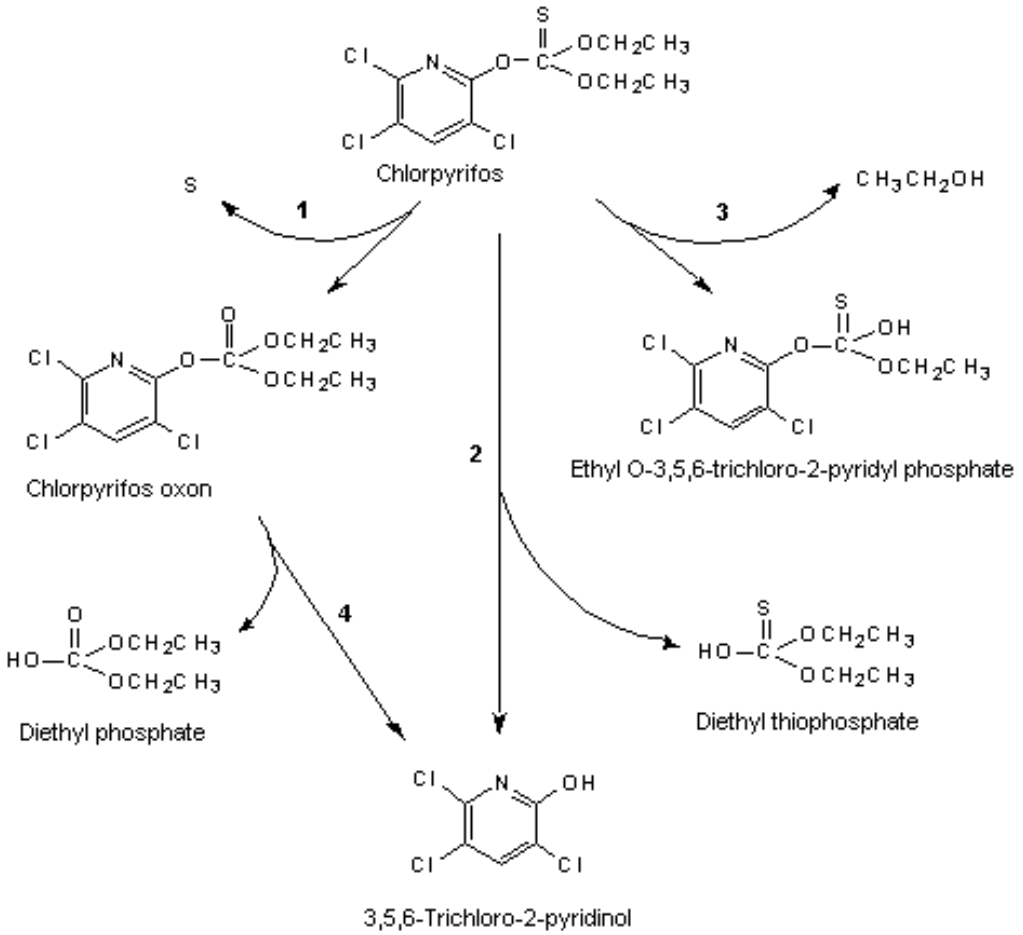

Fig. 2. Metabolic pathway of chlorpyrifos (From Smith et al.).

oral LD50 values indicate that chlorpyrifos is moderately toxic to small mammals on an acute oral basis. The most sensitive endpoint for the technical formulation, the rat LD50 of 118 mg/ kg is used estimate risk via direct effects mammals and indirect effects to birds, reptiles and terrestrial-phase amphibians. The oral LD50 for CPF in rats is 95 to 270 mg/kg [3, 37, 51]. The LD50 for CPF is 60 mg/kg in mice, 1000 mg/kg in rabbits, 32 mg/kg in chickens, 500 to 504 mg/kg in guinea pigs, and 800 mg/kg in sheep [44]. The dermal LD50 is greater than 2000 mg/kg in rats, and 1000 to 2000 mg/kg in rabbits. The 4-hour inhalation LC50 for CPF in rats is greater than 0.2 mg/L [3, 50]. CPF is moderately to very highly toxic to birds [53]. Acute LD50 values for technical grade chlorpyrifos available for avian species are with a range of LD50 values from 5,62 to 476 mg/kg. Its oral LD50 is 8,41 mg/kg in pheasants, 112 mg/kg in mallard ducks, 10 mg/kg in common pigeon, 21,0 mg/kg in house sparrows, and 32 mg/kg in chickens [53]. CPF is very highly toxic to freshwater fish, aquatic invertebrates and estuarine and marine organisms [13, 15]. Cholinesterase inhibition was observed in acute toxicity tests of fish exposed to very low concentrations of this insecticide. Application of concentrations as low as 0,01 pounds of active ingredient per acre may cause fish and aquatic invertebrate deaths [13, 15]. CPF toxicity to fish may be related to water temperature. The pesticide has the

potential to bioaccumulate in fish and other aquatic organisms and enter the aquatic food web. Due to its high acute toxicity and its persistence in sediments, chlorpyrifos may represent a hazard to sea bottom dwellers.

Animal studies confirm that CPF has higher systemic toxicity in neonates, with over an order of magnitude lower LD50 values than in adults [27, 31, 32, 54]. However, developing organisms recover more quickly from cholinesterase inhibition than do comparably exposed adults, largely due to the rapid synthesis of new cholinesterase molecules [31, 32, 48]. This discrepancy means either that cholinesterase inhibition is unrelated to developmental toxicity, or alternatively that even a brief period of cholinesterase inhibition is sufficient to disrupt development.

Chlorpyrifos is moderately toxic to humans but simultaneously it may be estimated that CPF causes thousands of deaths per year worldwide [16, 18, 40]. CPF is readily absorbed into the bloodstream through the gastrointestinal tract if it is ingested, through the lungs if it is inhaled, or through the skin if there is dermal exposure. In humans, CPF and its principal metabolites are eliminated rapidly. After a single oral dose, the half-life of c CPF in the blood appears to be about 1 day. Poisoning from CPF may affect the central nervous system, the cardiovascular system, and the respiratory system. It is also a skin and eye irritant [14, 17, 19]. Symptoms of acute exposure to CPF may include the following: numbness, tingling sensations, incoordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty breathing or respiratory depression, and slow heartbeat. Ingesting CPF orally through contaminated food containers or, in the case of children, putting objects of hands in their mouth after touching CPF, may cause similar symptoms. Very high doses may result in unconsciousness, incontinence, and convulsions or fatality. Persons with respiratory ailments, recent exposure to cholinesterase inhibitors, cholinesterase impairment, or liver malfunction are at increased risk from exposure to chlorpyrifos. Repeated or prolonged exposure to CPF may result in the same effects as acute exposure including the delayed symptoms. Other effects reported in workers repeatedly exposed include impaired memory and concentration, disorientation, severe depressions, irritability, confusion, headache, speech difficulties, delayed reaction times, nightmares, sleepwalking, and drowsiness or insomnia. An influenza-like condition with headache, nausea, weakness, loss of appetite, and malaise has also been reported [7, 26, 37].

There is no information at present to show that CPF either effects the ability of humans to reproduce or causes human birth defects. Also there is no evidence that chlorpyrifos is mutagenic and carcinogenic.

Neurotoxicity. Like the other OP insecticides, CPF inhibits the enzyme acetylcholinesterase (AChE), which hydrolyses acetylcholine, the neurotransmitter that activates cholinergic neurons. If acetylcholine is not inactivated immediately by the activity of AChE, it overstimulates the neurons, and tremors, convulsions and death can follow. Inhibition of AChE causes accumulation of acetylcholine at cholinergic synapses, leading to over-stimulation of muscarinic and nicotinic receptors. In addition, acetylcholine has important functions during brain and whole central nervous system (CNS) development [22, 23, 53]. Therefore inhibition of AChE by CPF and the resulting accumulation of acetylcholine may then conceivably disturb this development. Still, developing rats recover faster from AChE inhibition than adults, largely due to the fact that developing organisms have a rapid synthesis of new AchE molecules [31, 32, 49]. The majority of the neurological symptoms associated with CPF exposure result from its inhibition of acetylcholinesterase (AChE) and the subsequent cholinergic overstimulation. Common symptoms related to excessive cholinergic activity include headache, diaphoresis, nausea, vomiting, diarrhea, epigastric cramping, bradycardia, blurred vision, miosis,

bronchoconstriction and excess mucous secretions, pulmonary edema, dyspnea, muscle fasciculations, salivation, lacrimation, and urination [4, 22]. In adults and children, acute-duration inhalation exposure to unspecified concentrations of chlorpyrifos is associated with paresthesia and lightheadedness [20, 36, 41]. Headache is also a common occurrence [20, 41]. Additionally, in the Sherman (1995) report, acuteduration CPF exposure may produce signs of neurological toxicity weeks or months after the initial symptoms have resolved. For example, a family which became ill after an unspecified concentration of chlorpyrifos was applied in their home initially presented with headaches, nausea, and muscle cramps. However, numbness, paresthesia (most prominent in the legs), and memory impairment were reported by the family 1 month later. The children also showed a decline in scholastic performance that lasted for approximately 6 months. Neurological exams conducted 6 months post-exposure revealed mild short term memory loss on all routine mental status testing of recall of multiple objects. Neuropsychological testing was declined by the subjects, all other neurological exams were normal. Nerve conduction studies revealed low amplitude nerve action potentials in all family members. Motor and upper-extremity sensory nerve action potentials were normal. Sural nerve amplitudes in all but one family member had returned to normal 6 months later. Although inhalation was the most likely route of exposure, the family could also have been exposed dermally [21].

Clinical and experimental data indicate that OP-induced delayed neuropathies resulting from acute exposures to CPF require doses well in excess of the LD50. Studies in hens show that subchronic exposures at the maximum tolerated daily dose do not result in OP-induced delayed neuropathies. However, recovery from CPF toxicity when it does occur is unusually slow even when compared with other phosphorothioates. Furthermore, CPF has been shown to evoke learning deficits in rats after acute and repeated administration and to produce delayed sensory neuropathies in humans [50]. Moreover, the degree of AchE activity inhibiting by CPF does not correlate well with the onset of toxicity or the amount of exposure. These findings support new inquiries into additional mechanisms of CPF toxicity [50]. The results of the study of A.V. Terry indicate that the threshold for neurotoxic consequences (usually associated with higher doses of OPs) may be exceeded during repeated exposure to subthreshold doses of OPs, even for agents like CPF that have been considered moderately toxic or nonneuropathic. One potential mechanism for these observations is the prolonged inhibition of fast axonal transport. It is also important to note that CPF itself may have neurotoxic properties in the absence of conversion to its oxon or other metabolites. Although many of the toxic consequences of low-dose CPF administration were shown to be reversible after discontinuation, the cytotoxic action observed ex vivo suggests that under certain conditions of exposure, some actions may prove to be longer lasting [50].

In the study [38] authors tested the hypothesis that CPF and its metabolites alter the Ca^{2+}/cAMP response element binding protein (CREB), a critical molecule in brain development and cognitive function. They further tested the hypothesis that changes in CREB occur independent of AchE inhibition. Western blot analysis of lysates from primary cultures of cortical neurons exposed to CPF, CPF-oxon, or trichloropyridinol (TCP) for 1 h and cultures exposed to trichloropyridinol (TCP) for 7 days indicated that all exposures increased the level of the phosphorylated (activated) form of CREB (pCREB), without significant changes in total CREB or α-tubulin. Remarkably, pCREB in cortical neurons was elevated by 300–400% of control levels with estimated EC50s of 60 pM, and <30 pM for CPF, CPF-oxon, and TCP, respectively. AChE activity and cell viability were not affected by organophosphate concen-

trations that caused significant increases in pCREB (up to 100 nM, 100 pM, and 10 μM of CPF, CPF-oxon, and TCP, respectively). The level of pCREB in hippocampal neurons was also elevated after exposure to CPF, but pCREB in cultured astrocytes was not affected. Inclusion of the cytochrome P-450 inhibitor SKF-525A did not inhibit the effects of CPF on pCREB levels, indicating that metabolism of CPF to CPF-oxon was not necessary to cause the increase in pCREB. The increases in neuronal pCREB observed in this study provide biochemical evidence that CPF and its metabolites are active at critical sites within the nervous system at levels far below those required to inhibit AChE, which could explain many of the reported neurodevelopmental and behavioral changes attributed to CPF toxicity [38].

Emerging evidence, obtained largely through the use of rodents, suggests that acute or prolonged exposure to CPF and/or its metabolic product(s) may overtly injure the central nervous system or produce marked changes in neuronal function that persist after exposure has ceased, particularly during the early postnatal period [8, 9, 43, 45, 46, 54]. It seems that either developmental toxicity may be unrelated to AChE inhibition, or that even a brief period of AChE inhibition is sufficient to disrupt development [40, 42].

This field is under active investigation now. Studies are in progress to evaluate the importance of various factors and it is the object of a very thorough study. A lot of scientist, including Teodore Slotkin from USA, who is universally recognized as an authority on the subject, repeatedly demonstrated with his colleagues that CPF toxicity is not limited to cholinesterase inhibition alone but can act by other mechanisms. For example, *in vitro* and *in vivo* studies at three levels of development from DNA to the cell and the whole animal revealed that CPF is far more toxic than previously thought because of this wider range of activity [34]. CPF impairs the binding to DNA of nuclear transcription factors (AP-1 and Sp1) that modulate cell replication and differentiation. Therefore, CPF targets mammalian brain development through a combination of effects directed at cholinergic receptors and intracellular signaling cascades that are involved in cell differentiation.

It has been known, that CPF can induce neurobehavioral abnormalities during the second and third postnatal weeks in rat [14, 24, 29], corresponding to the neonatal stage in humans [51]. This period is outside the major phase of neurogenesis in most brain regions, but it is the time of peak gliogenesis and synaptogenesis. According to some authors developing glia have been found to be even more sensitive to chlorpyrifos than neurons [18, 19, 34, 35].

Deficits elicited by prenatal exposure to chlorpyrifos are evident even at exposures below the threshold for detectable AChE inhibition, i.e. far below the 70% inhibition of AChE required for systemic toxicity in adults [14, 37]. These findings suggest that mechanisms other than inhibition of AChE activity may, at least in part, be responsible for the developmental neurotoxicity of chlorpyrifos [13, 24, 29, 40].

Experiments of number of scientists have demonstrated the link between CPF and adverse neurodevelopmental sequelae in rodents. It was shown that subtoxic doses of CPF inhibit DNA synthesis, mitosis, neurite outgrowth, neural cell replication, neural cell differentiation and interfere with signaling cascades, including serotonergic, cholinergic and catecholaminergic pathways. CPF also inhibits glial cell replication, gliogenesis and glioma differentiation and disrupts the pattern of glial cell development *in vivo*. Because glial cells are targets of CPF at subtoxic doses, the vulnerability of the developing brain to CPF is increased [26, 32, 41].

Furthermore, recently Dr Anne Caughlan from the University of Washington has showned that CPF activates the ERK1/2 and p38 MAP kinases. Surprisingly, blocking ERK1/2 activation by the MEK inhibitor SL327 caused a small but statistically significant

inhibition of apoptosis, while blocking p38 with SB202190 significantly accelerated apoptosis induced by chlorpyrifos. This suggests a pro- and anti-apoptopic role for ERK1/2 and p38, respectively [11]. S. M. Mense from New York [26] also found that CPF significantly enhanced the levels of activated (phosphorylated) ERK1/2 . Because ERK1/2 is a key component of insulin signaling, this increase is consistent with the finding that targets of the insulin-signaling pathway were upregulated by the insecticide [26].

The aging brain shows selective neurochemical changes involving several neural cell populations. Increased brain metal levels have been associated with normal aging and a variety of neurodegenerative disorders. Copper is an important modulator of NMDA-receptor activity, zinc - of glutaminergic transmission. It is thus important to elucidate the mechanisms by which metal homeostasis of brain is maintained and how metals function in cellular processes, including neurotoxic damages.

In our laboratory we have studied levels of copper, zinc and iron in hippocampus, cerebellum and cerebral cortex of growing rats subjected to low doses of CPF. Our data demonstrates age-dependent changes of investigated metal levels in different brain regions. We have studied changes in activity of the antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase in hippocampus, cerebellum and cerebral cortex of growing rats subjected to low doses of CPF. We observed age-dependent and brain region-dependent changes in antioxidant enzymes activity. Simultaneously we studied the effect of the CPF action on maturation of hippocampal pyramidal cells and interneurons *in vitro* (cell culture). This includes study of the effects of CPF on the alterations and developmental changes in the neuronal and synaptic connections properties. We measured morphometric parameters of neurons including somatical, axonal and dendritic development, using for visualization different fluorescent proteins. A whole series of biochemical analysis of different parts of rat brain and our previous experiments with Morris water maze confirm the CPF neurotoxicity and at the same time impel for investigations on cellular and molecular level [1, 2].

Conclusions and perspectives of further inquiry. There can be no doubt about the importance of investigations of cellular and molecular mechanisms for toxicity of CPF including its developmental neurotoxicity. Agree with professor Slotkin the finding of a novel set of mechanisms underlying the developmental neurotoxicity of CPF sparked a wider degree of interest in the issue of OPs and brain development. Aforesaid questions suggest to be key points of future investigations. The main hypothesis is that CPF neurotoxicity predominantly is independent of AchE inhibition and that CPF induces apoptosis of developmental neurons. This suggestion is based on number of recent studies performed in well-known laboratories but this important and difficult task still confronts us [10, 11, 25, 33, 40, 44, 55].

1. *Салига Ю. Т.* Потенційна нейротоксичність хлорпірифосу і способи її вивчення // Медична хімія. 2009. Т. 11. № 4. С. 69–72.
2. *Салига Ю. Т., Слипанюк О. В.* Дослідження нейротоксичності хлорпірифосу у щурів за допомогою водного тесту Морріса та в умовах культури клітин гіпокампа // Фізіологічний журн. 2010. Т. 56. № 2. С. 49–50.
3. *Akhtar N., Srivastava M. K., Raisada R. B.* Transplacental disposition and teratogenic effects of chlorpyrifos in rats // J. Toxicol. Sci. 2006. Vol. 31. N 5. P. 521–527.
4. *Aldridge J. E., Seidler F. J., Meyer A.* et al. Serotonergic systems targeted by developmental exposure to chlorpyrifos: Eeffects during different critical periods // Environ. Health Perspect. 2003. Vol. 111. N 14. P. 1736–1743.

5. *Atterberry T. T., Burnett W. T., Chambers J. E.* Age-related differences in parathion and chlorpyrifos toxicity in male rats: target and nontarget esterase sensitivity and cytochrome P450-mediated metabolism // Toxicol. applied pharmacol. 1997. Vol. 147. P. 411–418.
6. *Barr D. B., Angerer J.* Potential uses of biomonitoring data: a case study using the organophosphorus pesticides chlorpyrifos and malathion // Environ. Health Perspect. 2006. Vol. 114. N 11. P. 1763–1769.
7. *Bjorling-Poulsen M., Andersen H. R., Grandjean P.* Potential developmental neurotoxicity of pesticides used in Europe // Environ. Health. 2008. Vol. 7. 50. doi: 10.1186/1476-069X-7-50.
8. *Buznikov G. A., Nikitina L. A., Bezuglov V. V.* et al. An invertebrate model of the developmental neurotoxicity of insecticides: effects of chlorpyrifos and dieldrin in sea urchin embryos and larvae // Environ. Health Perspect. 2001. Vol. 109. N 7. P. 651–661.
9. *Campbell C. G., Seidler F. J., Slotkin T. A.* Chlorpyrifos interferes with cell development in rat brain regions // Brain Res. Bull. 1997. Vol. 43. N 2. P. 179–189.
10. *Carvajal F., Sa´nchez-Amate M. C., Sa´nchez-Santed F., Cubero I.* Neuroanatomical targets of the organophosphate chlorpyrifos by c-fos immunolabeling // Toxicol. Sci. 2005. Vol. 84. P. 360–367.
11. *Caughlan A., Newhouse K., Namgung U., Xia Z.* Chlorpyrifos induces apoptosis in rat cortical neurons that is regulated by a balance between p38 and ERK/JNK MAP kinases // Toxicol. Sci. 2004. Vol. 78. N1. P. 125–134.
12. *Colborn T.* A case for revisiting the safety of pesticides: a closer look at neurodevelopment // Environ. Health Perspect. 2006. Vol. 114. N 1. P. 10–17.
13. *Costa L. G.* Current issues in organophosphate toxicology // Clinica Chimica Acta. 2006. Vol. 366. P. 1–13.
14. *Dam K., Seidler F. J., Slotkin T. A.* Chlorpyrifos exposure during a critical neonatal period elicits gender-selective deficits in the development of coordination skills and locomotor activity // Brain Res. Dev. Brain Res. 2000. Vol. 121. P. 179–187.
15. *Eskenazi B., Rosas L. G., Marks A. R.* et al. Pesticide toxicity and the developing brain // Basic & Clinical Pharmacol. Toxicol. Vol. 102. P. 228–236.
16. *Eyer F., Roberts D. M., Buckley N. A.* et al. Extreme variability in the formation of chlorpyrifos oxon (CPO) in patients poisoned by chlorpyrifos (CPF) // Biochem. Pharmacol. 2009. Vol. 78. N 5. P. 531–537.
17. *Garcia S. J., Seidler F. J., Slotkin T. A.* Developmental neurotoxicity elicited by prenatal or postnatal chlorpyrifos exposure: effects on neurospecific proteins indicate changing vulnerabilities // Environ. Health Perspect. 2003. Vol. 111. N 3. P. 297–303.
18. *Garcia S. J., Seidler F. J., Crumpton T. L., Slotkin T. A.* 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. Vol. 891. P. 54–68.
19. *Garcia S. J., Seidler F. J., Qiao D., Slotkin T. A.* Chlorpyrifos targets developing glia: effects on glial fibrillary acidic protein // Brain Res. Dev. Brain Res. 2002. Vol. 133. P. 151–161.
20. *Hertz-Picciotto I., Park H. Y., Dostal M.* et al. Prenatal exposures to persistent and nonpersistent organic compounds and effects on immune system development // Basic Clinical Pharmacol. Toxicol. 2008. Vol. 102. P. 146–154.
21. *Hunter D. L., Marshall R. S., McDaniel K. L., Phillips P. M.* Age- and gender-related differences in the time course of behavioral and biochemical effects produced by oral

chlorpyrifos in rats // Toxicol. applied pharmacol. 1998. Vol. 149. P. 107–119.
22. *Lauder J. M.* Roles for neurotransmitters in development: possible interaction with drugs during the fetal and neonatal periods // Prog. Clin. Biol. Res. 1985. Vol. 163. P. 375–380.
23. *Lauder J. M., Schambra U. B.* Morphogenetic roles of acetylcholine // Environ. Health Perspect. 1999. Vol. 107. N 1. P. 65–69.
24. *Levin E. D., Addy N., Nakajima A.* et al. Persistent behavioral consequences of neonatal chlorpyrifos exposure in rats // Brain. Res. Dev. Brain Res. 2001. Vol. 130. P. 83–89.
25. *Li1 Q., Kawada T.* The mechanism of organophosphorus pesticide-induced inhibition of cytolytic activity of killer cells // Cellular Molecular Immunol. 2006. Vol. 3. N 3. P. 171–178.
26. *Mense S. M., Sengupta A., Lan C.* et al. The common insecticides cyfluthrin and chlorpyrifos alter the expression of a subset of genes with diverse functions in primary human astrocytes // Toxicol. Sci. 2006. Vol. 93. N 3. P. 125–135.
27. *Meyer A., Seidler F. J., Cousins M. M., Slotkin T. A.* Developmental Neurotoxicity Elicited by Gestational Exposure to Chlorpyrifos: When Is Adenylyl Cyclase a Target? // Environ. Health Perspect. 2003. Vol. 111. N 16. P. 1871–1876.
28. *Mitra N. K., Siong H. H., Nadarajah V. D.* Evalution of neurotoxicity of repeated dermal application of chlorpyrifos on hippocampus of adult mice // Ann. Agric. Environ. Med. 2008. Vol. 15. P. 211–216.
29. *Moser V. C.* Dose-response and time-course of neurobehavioral changes following oral chlorpyrifos in rats of different ages // Neurotoxicol. Teratol. 2000. Vol. 22. P. 713–723. (53pn).
30. *Needham L. L.* Assessing exposure to organophosphorus pesticides by biomonitoring in epidemiologic studies of birth outcomes // Environ. Health Perspect. 2005. Vol. 113. N 4. P. 494–498.
31. *Pope C. N., Chakraborti T. K.* Dose-related inhibition of brain and plasma cholinesterase in neonatal and adult rats following sublethal organophosphate exposures // Toxicol. 1992. Vol. 73. P. 35–43.
32. *Pope C. N., Chakraborti T. K., Chapman M. L.* et al. Comparison of in vivo cholinesterase inhibition in neonatal and adult rats by three organophosphorothioate insecticides // Toxicol. 1991. Vol. 68. P. 51–61.
33. *Prendergast M. A., Self R. L., Smith K. J. e*t al. Microtubule-associated targets in chlorpyrifos oxon hippocampal neurotoxicity // Neurosci. 2007. Vol. 146. N 1. P. 330–339.
34. *Qiao D., Seidler F. J., Slotkin T. A.* Developmental neurotoxicity of chlorpyrifos modeled in vitro: comparative effects of metabolites and other cholinesterase inhibitors on DNA synthesis in PC12 and C6 cells // Environ. Health Perspect. 2001. Vol. 109. P. 909–913.
35. *Qiao D., Seidler F. J., Tate C. A.* et al. Fetal chlorpyrifos exposure: adverse effects on brain cell development and cholinergic biomarkers emerge postnatally and continue into adolescence and adulthood // Environ. Health Perspect. 2003. Vol. 111. P. 536–544.
36. *Ricceri L., Markina N., Valanzano A.* et al. Developmental exposure to chlorpyrifos alters reactivity to environmental and social cues in adolescent mice // Toxicol. Appl. Pharmacol. 2003. Vol. 191. P. 189–201.
37. *Ricceri L., Venerosi A., Capone F.* et al. Developmental neurotoxicity of organophosphorousn pesticides: fetal and neonatal exposure to chlorpyrifos alters sex-specific behaviors at adulthood in mice // Toxicol. Sci. 2006. Vol. 93. N 3. P. 105–113.
38. *Schuh R. A., Lein P. J., Beckles R. A., Jett D. A.* Noncholinesterase mechanisms of

chlorpyrifos neurotoxicity: altered phosphorylation of Ca^{2+}/cAMP response element binding protein in cultured neurons // Toxicol. Appl. Pharmacol. 2002. Vol. 182. P. 176–185.

39. *Shemer H., Sharpless C. M., Linden K. G.* Photodegradation of 3,4,6-trichloro-2-pyridinol in aqueous solution // Water Air Soil Pollut. 2005. Vol. 168. P. 145–155.
40. *Slotkin T. A.* Cholinergic systems in brain development and disruption by neurotoxicants: nicotine, environmental tobacco smoke, organophosphates // Toxicol. Appl. Pharmacol. 2004. Vol. 198. P. 132–151.
41. *Slotkin T. A.* Developmental cholinotoxicants: nicotine and chlorpyrifos // Environ. Health Perspect. 1999. Vol. 107. N 1. P. 71–80.
42. *Slotkin T. A.* Nicotine and the adolescent brain: insights from an animal model // Neurotoxicol. Teratol. 2002. Vol. 24. P. 369–384.
43. *Slotkin T. A., Greer N., Faust J.* et al. Effects of maternal nicotine injections on brain development in the rat: ornithine decarboxylase activity, nucleic acids and proteins in discrete brain regions // Brain Res. Bull. 1986. Vol. 17. P. 41–50.
44. *Slotkin T. A., Levin E. D., Seidler F. J.* Comparative developmental neurotoxicity of organophosphate insecticides: effects on brain development are separable from systemic toxicity // Environ. Health Perspect. 2006. Vol. 114. P. 746–751.
45. *Slotkin T. A., MacKillop E. A., Ryde I. T., Seidler F. J.* Ameliorating the developmental neurotoxicity of chlorpyrifos:a mechanisms-based approach in PC12 cells // Environ. Health Perspect. 2007. Vol. 115. N 9. P. 1306–1313.
46. *Slotkin T. A., Seidler F. J., Wu C.* Ultraviolet Photolysis of Chlorpyrifos: Developmental Neurotoxicity Modeled in PC12 Cells // Environ. Health Perspect. 2009. Vol. 117. P. 338–343.
47. *Slotkin T. A., Seidler F. J.* Comparative developmental neurotoxicity of organophosphates in vivo: Transcriptional responses of pathways for brain cell development, cell signaling, cytotoxicity and neurotransmitter systems // Brain Res. Bull. 2007. Vol. 72. P. 232–274.
48. *Song X., Seidler F. J., Saleh J. L.* et al. Cellular mechanisms for developmental toxicity of chlorpyrifos: targeting the adenylyl cyclase signaling cascade // Toxicol. Appl. Pharmacol. 1997. Vol. 145. P. 158–174.
49. *Song X., Violin J. D., Seidler F. J., Slotkin T. A.* Modeling the developmental neurotoxicity of chlorpyrifos in vitro: macromolecule synthesis in PC12 cells // Toxicol. Appl. Pharmacol. 1998. Vol. 151. P. 182–191.
50. *Terry A. V., Stone J. D., Buccafusco* J. J. et al. Repeated exposures to subthreshold doses of chlorpyrifos in rats:hippocampal damage, impaired axonal transport, and deficits in spatial learning // J. Pharmacol. Experimental Therapeutics. 2003. Vol. 305. N 1. P. 375–384.
51. *Vidair C. A.* Age dependence of organophosphate and carbamate neurotoxicity in the postnatal rat: extrapolation to the human // Toxicol. Appl. Pharmacol. 2004. Vol. 196. P. 287–302.
52. *Wauchope R. D., Buttler T. M., Hornsby A. G.* et al. SCS/ARS/CES Pesticide properties database for environmental decisionmaking // Rev. Environ. Contam. Toxicol. 1992. Vol. 123: 1-157. P. 5–20.
53. *Whitaker-Azmitia P. M.* Role of serotonin and other neurotransmitter receptors in brain development: basis for developmental pharmacology // Pharmacol. Rev. 1991. Vol. 43. P. 553–561.
54. *Whitney K. D., Seidler F. J., Slotkin T. A.* 1995. Developmental neurotoxicity of chlorpyrifos: cellular mechanisms // Toxicol. Appl. Pharmacol. 1995. Vol. 134. P. 53–62.

55. *Yang D., Howard A., Bruun D.* et al. Lein Chlorpyrifos and Chlorpyrifos-Oxon Inhibit Axonal Growth by Interfering with the Morphogenic Activity of Acetylcholinesterase // Toxicol. Appl. Pharmacol. 2008. Vol. 228. N 1. P. 32–41.

ОЦІНКА БІОЛОГІЧНОГО ВПЛИВУ ХЛОРПІРИФОСУ І ДЕЯКІ АСПЕКТИ ЙОГО НЕЙРОТОКСИЧНОСТІ

Ю. Салига

Інститут біології тварин НААН України
вул. В.Стуса, 38, Львів 79034, Україна
e-mail: yursalyha@yahoo.com

У даній роботі дається загальний огляд хлорпірифосу – одного з найпоширеніших у світі фосфорорганічних пестицидів. Цей огляд не вичерпує всіх проблем біохімії хлорпірифосу. Ці питання детально обговорювалися у різних оглядах і тому лише коротко будуть викладені тут. Було зроблено спробу дати огляд численних літературних даних, що стосуються механізмів нейротоксичності хлорпірифосу. Давно відомо, що хлорпірифос є інгібітором ацетилхолінестерази і що висока чутливість холінестераз до фосфорорганічних інгібіторів робить ці інгібітори надзвичайно токсичними для центральної нервової системи. Сьогодні хлорпірифос продовжує викликати значний дослідницький інтерес. За останні п'ять років з'явилися деякі нововведення у питаннях, що стосуються механізмів нейротоксичності хлорпірифосу, зокрема, було неодноразово продемонстровано, що його токсичність не лімітується інгібуванням холінестерази, а може мати інші механізми дії. Тут зведено наявну літературу, що стосується даного питання.

Ключові слова: мозок, центральна нервова система, хлорпірифос, холінестераза, нейротоксичність, фосфорорганічні інсектициди, пестициди.

ОЦЕНКА БИОЛОГИЧЕСКОГО ВЛИЯНИЯ ХЛОРПИРИФОСА И НЕКОТОРЫЕ АСПЕКТЫ ЕГО НЕЙРОТОКСИЧНОСТИ

Ю. Салыга

Институт биологии животных НААН Украины
ул. В.Стуса, 38, Львов 79034, Украина
e-mail: yursalyha@yahoo.com

В данной работе дается общий обзор хлорпирифоса – одного из самых распространенных в мире фосфорорганических пестицидов. Этот обзор не исчерпывает всех проблем биохимии хлорпирифоса. Этот вопрос подробно обсуждался в различных обзорах и поэтому лишь кратко будет изложен здесь. Была предпринята попытка дать обзор многочисленных литературных данных, касающихся механизмов нейротоксичности хлорпирифоса. Давно известно, что хлорпирифос является ингибитором ацетилхолинэстеразы и что высокая чувствительность холинэстераз к фосфорорганическим ингибиторам делает эти ингибиторы в высшей степени токсичными для центральной нервной системы. Сегодня хлорпирифос продолжает вызывать значительный исследовательский интерес. За последние пять лет появились некоторые новшества в вопросах, касающихся механизмов нейротоксичности хлорпирифоса, в частности, было неоднократно продемонстрировано, что токсичность хлорпирифоса не лимитируется ингибированием холинэстеразы, а может обладать другими механизмами воздействия. Здесь сведена существующая литература, касающаяся этого вопроса.

Ключевые слова: мозг, центральная нервная система, хлорпирифос, холинэстераза, нейротоксичность, фосфорорганические инсектициды, пестициды.