Acetylcholinesterase as a potential target of acute neurotoxic effects of lindane in rats

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Abstract. The aim of our study was to investigate the possible involvement of acetylcholinesterase (AchE) in mediating the early phase of acute lindane neurotoxicity in rats. Male Wistar rats ($n = 48$) were divided into following groups: 1. control, saline-treated group; 2. dimethylsulfoxide-treated group; 3. group that received lindane dissolved in dimethylsulfoxide, in a dose of 8 mg/kg intraperitoneally. Eight animals from each group were sacrificed 0.5 and 4 h after treatment and brain samples were prepared for further analysis. AchE activity (mitochondrial and synaptosomal fraction) was determined in cerebral cortex, thalamus, hippocampus and nc. caudatus spectrophotometrically. A significant increase in mitochondrial AchE activity was detected in cortex and nc. caudatus of lindane-treated animals 0.5 h after administration ($p < 0.05$). This rise was sustained in nc. caudatus within 4 h after treatment ($p < 0.05$). In contrast, activity of synaptosomal AchE fraction was significantly increased only in thalamus 4 h after lindane administration ($p < 0.05$). An increase in AchE activity may be involved in mediating acute neurotoxic effects of lindane, at least in some brain structures in rats.

Key words: Acetylcholinesterase — Lindane — Neurotoxicity — Rats

Introduction

Lindane (γ-hexachlorocyclohexane) is an organochlorine pesticide used extensively in agriculture and in human and veterinary medicines (Ntow 2001; Katsumata and Katsumata 2003; Mills and Yang 2003; Muscat et al. 2003; Kalantzi et al. 2004; Radosavljević et al. 2008). This environmentally persistent xenobiotic was reported to exert neurotoxic (Sahoo et al. 2000; Parmar et al. 2003; Sahaya et al. 2007), cardiovascular (Anand et al. 1995), gastrointestinal (Moreno et al. 1996), renal (Dietrich and Swenberg 1990), respiratory (Brown 1988), reproductive (Srivastava and Raizada 2000),

hematological (Rauch et al. 1990), musculoskeletal (Hong and Boorman 1993), endocrine (Beard and Rawlings 1999), metabolic (Agrawal et al. 1995; Mladenović et al. 2008), immunological and lymphoreticular (Hong and Boorman 1993), hepatotoxic (Radosavljević et al. 2008), and proconvulsive effects (Martinez and Martinez-Conde 1995; Parmar et al. 2003; Kaminski et al. 2004; Vučević et al. 2008). It has been postulated that lindane achieves its behavioral effects and neurotoxicity through several mechanisms, like interfering with γ-aminobutiryc acid (GABA), Na^+,K^+-ATPase, Mg^{2+}-ATPase and acetylcholinesterase (AchE), inducing imbalance of the central monoaminergic systems, alteration of cerebral glucose uptake, induction of brain cytochrome P450s, alteration of neurotransmitter levels, etc. (Anand et al. 1998; Rivera et al. 1998; Sahoo et al. 2000; Parmar et al. 2003).

Changes in levels of brain norepinephrine and serotonin (Rivera et al. 1991) have been reported in rats administered

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acute oral doses of lindane. Decreased dopamine levels were seen in rats treated by gavage with 10 doses totaling 60 mg lindane/kg (half the LC_{50}) over a period of 30 days (Martinez and Martinez-Conde 1995). Increase in the levels of brain catecholamines, particularly norepinephrine and dopamine, and associated signs of toxicity such as mild tremor, lacrimation, salivation, and dyspnea were observed in female rats given oral doses of 100 mg/kg/day of technical-grade lindane for 7 days (Raizada et al. 1993). The activity of monoamine oxidase (MAO) in the cerebrum showed a marginal decrease, while the cerebellum and spinal cord indicated a significant increase and cdecrease in MAO, respectively. Rats treated with 20 mg technical-grade lindane/kg/day in food for 90 days exhibited increased GABA levels, increased glutamate decaarboxylase activity, and decreased glutamate levels in the brain (Nagaraya and Desiraju 1994).

Neurotransmission is regulated mainly by the release of neurotransmitter from presynaptic nerve terminals, the accumulation of neurotransmitter at the synaptic clefts, and the function of the postsynaptic receptors. Acetylcholine (Ach), in particular, is known to be rapidly hydrolyzed by AchE (Cooper et al. 2003). The duration of action of this major neurotransmitter in peripheral and central cholinergic system at the synaptic clefts depends critically on AchE activity (Prado et al. 2002). AchE belongs to a family of enzymatic proteins distributed in cholinergic neurons and widely throughout the body mainly in cholinergic nerves and erythrocytes (Prado et al. 2002; Cooper et al. 2003). Apart from its catalytic function in hydrolyzing Ach, AchE affects cell proliferation, differentiation and responses to various insults, including stress (Grisaru et al. 1999). It also performs nonenzymatic, trophic (e.g. stimulation of neuritogenesis and remodeling) and neuromodulatory (promotion of long-term functional changes in the central nervous system) functions (Gralewicz 2006).

Despite existing knowledge, the data on the AchE role in early phase of acute lindane intoxication is not reported in the curently available literature. Taking into account the absence of studies showing the influence of AchE activity on lindane neurotoxic effects, this study was aimed to investigate the possible involvement of AchE in mediating the early phase of acute neurotoxicity of this pesticide in rats.

Materials and Methods

Animals

Experiments were performed on adult male Wistar rats, weighing 170–200 g, raised at Military Medical Academy Breeding Laboratories, Belgrade. Animals were kept under standard controlled laboratory conditions (temperature 22 ± 2°C, relative humidity 50% and 12/12 h light/dark cycle with light switched on at 9 a.m.), and were housed individually with free access to standard pelleted food and tap water. All experimental procedures were in full compliance with The European Council Directive (86/609/EEC) and approved by The Ethical Committee of the University of Belgrade (Permission No. 298/5-2).

All animals ($n = 48$) were divided into following groups: 1. control, saline-treated ($n = 16$); 2. DMSO-treated ($n = 16$); 3. group that received lindane in a dose of 8 mg/kg intraperitoneally (i.p.) ($n = 16$). Before administration, lindane (Sigma-Aldrich Chemical Co., U.S.A.) was dissolved in dimethylsulfoxide (DMSO; Sigma-Aldrich Chemical Co., U.S.A.) and injected in a volume of 0.5 ml/kg body weight. The dose of 8 mg/kg was chosen since in previous studies convulsions were found to appear in rats after acute exposure to this dose (Mladenović et al. 2007). Eight animals from each group were sacrificed by decapitation 0.5 and 4 h after treatment.

Biochemical analysis

After decapitation the heads were immersed into liquid nitrogen and stored at –80°C. For the determination of enzyme activity crude synaptosomal and mitochondrial fractions of specific brain regions were prepared according to the method of Whittaker and Barker (1972) with the omission of purification step through sucrose gradient. Isolation of cerebral cortex, hippocampus, thalamus and nc. caudatus from individual animals was done quickly on ice. Isolated tissue was homogenized in ice-cold buffer, pH 7.0, containing 0.25 mmol/l sucrose, 0.1 mmol/l EDTA, 50 mmol/l K-Na phosphate buffer. Homogenates were centrifuged twice at $1000 \times g$ for 15 min at 4°C. The supernatant was further centrifuged at $20,000 \times g$ for 20 min. Supernatant obtained by this procedure represents crude synaptosomal fraction containing membrane vesicles (microsomes) from smooth and rough endoplasmic reticulum, Golgi and plasma membrane and all of the soluble components of the cytoplasm. The pellet was resuspended in deionized water and left for 60 min at +4°C. Finally, resuspended pellet was centrifuged at $1700 \times g$ for 15 min. Obtained supernatant represents crude mitochondrial fraction containing mitochondria, lysosomes, peroxisomes, Golgi membranes and some rough endoplasmic reticulum. Crude synaptosomal and crude mitochondrial fractions were stored at –80°C.

AchE activity, expressed as mol/mg prot./min $\times 10^{-4}$ was assayed by spectrophotometric method of Ellman et al. (1961), using acetylthiocholine iodide as a substrate in homogenates of cerebral cortex, hippocampus, thalamus and nc. caudatus. Each sample was taken from one animal and assayed in duplicate. The rate of hydrolysis of acetylthiocholine iodide was measured at 412 nm through the release

of the thiol compound which, when reacted with 5,5`-dithio-bis 2-nitrobenzoic acid (DTNB), produced the absorbing compound thionitrobenzoic acid.

Statistical analysis

For statistical purposes, one-way ANOVA was followed by Newman-Kuels test for multiple comparisons taking $p < 0.05$ as the level of significance. All the results were expressed as means ± SD.

Results

No significant changes in AchE activity were detected in any brain region in control group 0.5 or 4 h after treatment (data not shown). In addition, administration of DMSO did not induce any significant change in the activity of both AchE izoenzymes within 0.5 or 4 h in comparison with control group ($p > 0.05$) (data not shown).

The AchE activity in the crude mitochondrial fraction of cerebral cortex was significantly increased in the lindane-treated animals 0.5 h after administration of this pesticide (0.272 ± 0.018 mol/mg prot./min × 10^{-4}) ($p < 0.05$) in comparison with its basal activity measured in control group (0.191 ± 0.015 mol/mg prot./min × 10^{-4}) (Fig. 1A).

There were no significant changes in the AchE activity in the crude mitochondrial and synaptosomal fraction of hippocampus in the lindane-treated animals in comparison with its basal activity found in control group (Fig. 1B).

A significant rise in synaptosomal AchE activity was detected in thalamus of animals that received lindane (0.388 ± 0.105 mol/mg prot./min × 10^{-4}) ($p < 0.05$), 4 h after its administration in comparison with control value (0.282 ± 0.076 mol/mg prot./min × 10^{-4}) (Fig. 1C).

The AchE activity in the crude mitochondrial fraction of nc. caudatus was significantly increased in lindane-injected animals 0.5 h (4.011 ± 0.648 mol/mg prot./min × 10^{-4}) ($p < 0.05$) and 4 h (4.336 ± 0.855 mol/mg prot./min × 10^{-4}) ($p < 0.05$) after intraperitoneal application of this convulsant when compared to control value (3.117 ± 0.083 mol/mg prot./min × 10^{-4}) (Fig. 1D).

Discussion

The mode of action by which lindane alters neurotransmitter equilibrium in the brain and cause neurotoxicity is still an incompletely known territory. From a toxicological point of view, our results represent important findings, because the influence of lindane on the AchE activity has not been examined in the *in vitro* and *in vivo* experiments.

Our investigation has shown that in the lindane-treated animals 0.5 h after administration of this pesticide the AchE activity in the crude mitochondrial fraction of cerebral cortex was significantly increased ($p < 0.05$) (Fig. 1A). On the other hand, investigation reported by Gopal et al. (1992) concerning assessment of brain biochemistry has shown effects that reduced total brain ATPase and AchE activity. Another similar investigations have shown that lindane neurotoxicity include alterations in the activities of various enzymes, including Na$^+$, K$^+$-ATPase, Mg^{2+}-ATPase and AchE (Raizada et al. 1993; Martinez and Martinez-Conde 1995; Anand et al. 1991, 1998; Rivera et al. 1998; Sahoo et al. 2000; Parmar et al. 2003). Additionally, it is known that acute administration of high doses of aluminium significantly reduces AchE activity in mitochondrial and microsomal fractions of the cortex of gerbil brain (Mićić and Petronijević 2000).

As it is previously mentioned, any studies have not been conducted on lindane mechanism involving cholinergic system. In our experiment there was no significant difference between the AchE activity in the crude mitochondrial and synaptosomal fraction of hippocampus in the lindane-treated animals 0.5 and 4 h after treatment with this substance (Fig. 1B). On the other hand, AchE activity in mitochondrial and microsomal fractions of the hippocampus of gerbil brain was significantly decreased after acute administration of high doses of aluminium (Mićić and Petronijević 2000).

Ach has an important role in peripheral and central nervous system. Apart from glutamatergic, hippocampus consists of cholinergic neurons that form rich connections with numerous brain regions (Hossain et al. 2004). Besides, a large portion of the septohippocampal projection is GABAergic (Amaral and Kurz 1985) and the septal GABAergic afferents terminate exclusively on hippocampal interneurons (Freund and Antal 1988). Cholinergic activation depresses excitatory responses by increasing GABA release from interneuron terminals (Pitler and Alger 1992). It is also known that events included in the synthesis and release of Ach require acetyl coenzyme A and energy from the mitochondria in nerve terminals. It is possible that lindane caused a disturbance of mitochondrial respiration in the hippocampus which led to non significant changes in the activities of AchE fractions in the hippocampus of investigated rats in the present study.

The primary target for lindane action was suggested to be GABA receptor. Lindane binds to the picrotoxin site of ionotropic GABA$_A$ receptor, thus inhibiting its function and enhancing excitatory neurotransmission in the central nervous system. This effect was confirmed by suppression of lindane-induced convulsions by various GABA$_A$ agonists and their exacerbation by GABA$_A$ antagonists (Martinez and Martinez-Conde 1995; Anand et al. 1998). However, the effect of the lindane in the present experiment may have affected both the cholinergic and GABAergic interneurons which interact within the hippocampus. It is possible that
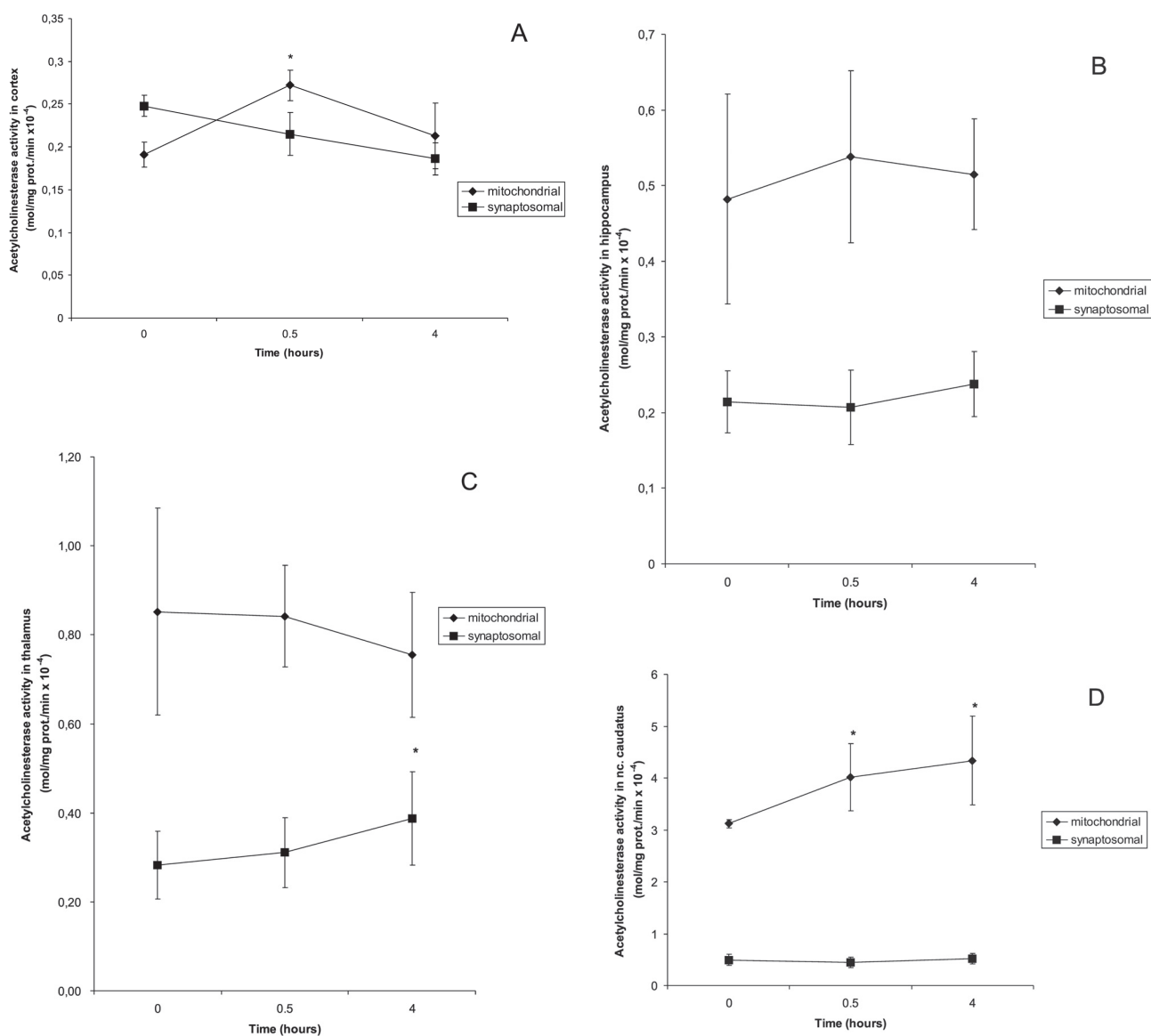
Figure 1. Acetylcholinesterase (AchE) activity in cerebral cortex (A), hippocampus (B), thalamus (C) and nc. caudatus (D) in rats 0, 0.5 and 4 h after lindane (8 mg/kg, i.p) administration. AchE activities measured in control group were considered to be its basal activities (0 h). Data are means ± SD, * $p < 0.05$ (one way ANOVA and Newman-Kuels test) in comparison with basal activity (0 h).

a lindane increases the release of Ach *via* affecting sodium channels of cholinergic nerves and also decreases the release of Ach via increasing GABA release that may, in turn, inhibit the cholinergic excitement. Additionally, Soderlund et al. (2002) clearly demonstrated that, unlike insects, mammals have multiple sodium channel isoforms that vary in their biophysical and pharmacological properties, including their differential sensitivity to pesticides. The literature, also, describe hippocampal differences in sensitivity to pesticide action in comparison with other regions of the brain (Soderlund et al. 2002; Hossain et al. 2004, 2005; Gralewicz

2006; Trevisan et al. 2008). Namely, with low doses, and after a short period of exposure, malathion induces an up-regulation of hippocampal and cortical AchE activity (Trevisan et al. 2008).

The inhibition of AchE activity in target tissues is often taken as an indication of pesticide intoxication (Kwong 2002), resulting in the hyper-stimulation of cholinergic receptors (Carr et al. 2001). In the hippocampus the activity of the antioxidant enzymes studied correlates positively with AchE activity increase (Trevisan et al. 2008). In relation to, oxidative stress has been hypothesized as an alternative

pathway for organophosphate toxicity, by either increasing reactive oxygen species production or decreasing antioxidant defenses. It is known that oxidative perturbation can be an alternative pathway for malathion toxicity in the central nervous system (Trevisan et al. 2008). Besides, the absence of AchE inhibition, suggests that the cholinergic system may not be directly involved in the toxic effects of malathion at repeated low doses. Furthermore, cerebral cortex and hippocampus were able to increase AchE, which may be considered an adaptive response. Among alternative targets, reduction of antioxidant protection, especially in the cerebral cortex, is a possible alternative route for malathion toxicity (Trevisan et al. 2008). Similarly, lindane has also been shown to be a strong oxidant causing free radical generation in tissues including brain through lipid peroxidation. This oxidant generation is accompanied by alterations in antioxidant capacity of the brain, too (Sahoo et al. 2000; Sahaya et al. 2007). It cannot be excluded that alteration of AchE activity is a contributing factor in lindane toxicity in our experiment.

A significant rise ($p < 0.05$) in synaptosomal AchE activity was detected in thalamus of animals 4 h after lindane administration (Fig. 1C). On the other hand, acute administration of high doses of aluminium significantly reduces AchE activity in mitochondrial and microsomal fractions of the thalamus of gerbil brain (Mićić and Petronijević 2000).

As shown in Fig. 1D, the AchE activity in the crude mitochondrial fraction of nc. caudatus was significantly increased ($p < 0.05$) in lindane-injected animals.

AchE was found in various molecular forms, depending on alternative splicing of its transcripts and association with structural proteins. Tetramers of the "tailed" variant (AchE-T), which are anchored in the cell membrane of neurons by the PRiMA (Proline Rich Membrane Anchor) protein, constitute the main form of AchE in the mammalian brain. In the mouse brain, stress and anticholinesterase inhibitors have been reported to induce expression of the unspliced "readthrough" variant (AchE-R) mRNA which produces a monomeric form (Perrier et al. 2005). Besides, AchE responses (cell proliferation, differentiation, responses to various insults, including stress) are at least in part specific to the three C-terminal variants of AchE, which are produced by alternative splicing of the single AchE gene. "Synaptic" AchE-S constitutes the principal multimeric enzyme in brain and muscle; soluble, monomeric "readthrough" AchE-R appears in embryonic and tumor cells and is induced under psychological, chemical and physical stress; and glypiated dimers of erythrocytic AchE-E associate with red blood cell membranes. It has been postulated that the homology of AchE to the cell adhesion proteins, gliotactin, glutactin and the neurexins, which have more established functions in nervous system development, is the basis of its morphogenic functions (Grisaru et al. 1999). Taking these facts into consideration, results of our study also suggest

that competition between AchE variants and their homologs on interactions with the corresponding protein partners is possible in investigated brain structures in rats during lindane-induced neurotoxicity.

On the basis of our study, it can be concluded for the first time the possible involvement of AchE activity in mediating the early phase of acute lindane neurotoxicity in rats. However, the exact mechanism of AchE influence on lindane animal model of acute neurotoxicity will require further investigation.

References

Agrawal D., Subramoniam A., Afaq F. (1995): Influence of hexachlorocyclohexane on phosphoinositides in rat erythrocyte membranes and brain. Toxicology **95**, 135–140

Amaral D. G., Kurz J. (1985): An analysis of the origins of the cholinergic and noncholinergic septal projections to the hippocampus formation of the rat. J. Comp. Neurol. **240**, 35–59

Anand M., Agrawa A. K., Rehmani B. N. H. (1998): Role of GABA receptor complex in low dose lindane (HCH) induced neurotoxicity: Neurobehavioural, neurochemical and electrophysiological studies. Drug. Chem. Toxicol. **21**, 35–46

Anand M., Gupta G. S. D., Gopal K. (1991): Influence of dietary protein deficiency on EEG neurotransmitters and neurobehaviour after chronic exposure to HCH. Toxicol. Environ. Chem. **34**, 1–11

Anand M., Meera P., Kumar R. (1995): Possible role of calcium in the cardiovascular effects of prolonged administration of gamma-HCH (Lindane) in rats. J. Appl. Toxicol. **15**, 245–248

Beard A. P., Rawlings N. C. (1999): Thyroid function and effects on reproduction in ewes exposed to the organochlorine pesticides lindane or pentachlorophenol (PCP) from conception. J. Toxicol. Environ. Health **58**, 509–530

Brown D. (1988): Lindane: 13 week dermal toxicity study (with interim kill and recovery period) in the rat. Hazelton UK, North Yorkshire, England. HUK report no. 5757–5802

Carr R. L., Chambers H. W., Guarisco J. A., Richardson J. R., Tang J., Chambers J. E. (2001): Effects of repeated oral postnatal exposure to chlorpyrifos on open-field behavior in juvenile rats. Toxicol. Sci. **59**, 260–267

Cooper J. R., Bloom F. E., Roth R. H., (2003): Acetylcholine. The Biochemical Basis of Neuropharmacology. Oxford University Press, New York

Dietrich D. R., Swenberg J. A. (1990): Lindane induces nephropathy and renal accumulation of α2μ-globulin in male but not in female Fischer 344 rats or male NBR rats. Toxicol. Letters **53**, 179–181

Ellman G. L., Courtney K. D., Andres V. Jr., Feather-Stone R. M. (1961): A new and rapid colorimetric determination of

acetylcholinesterase activity. Biochem. Pharmacol. **7**, 88–95

Freund T. F., Antal M. (1988): GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. Nature **336**, 170–173

Gopal K., Anand M., Khanna R. N. (1992): Some neurotoxicological consequences of hexachlorocyclohexane (HCH) stress in rats fed on protein deficient diet. Toxicol. Environ. Chem. **36**, 57–63

Gralewicz S. (2006): Possible consequences of acetylcholinesterase inhibition in organophosphate poisoning. Discussion continued. Med. Pr. **57**, 291–302 (in Polish)

Grisaru D., Sternfeld M., Eldor A., Glick D., Soreq H. (1999): Structural roles of acetylcholinesterase variants in biology and pathology. Eur. J. Biochem. **264**, 672–686

Hong H. L., Boorman G. A. (1993): Residual myelotoxicity of lindane in mice. Fundam. Appl. Toxicol. **21**, 500–507

Hossain M. M., Suzuki T., Sato I., Takewaki T., Suzuki K., Kobayashi H. (2004): The modulatory effect of pyrethroids on acetylcholine release in the hippocampus. Neurotoxicology **25**, 825–833

Hossain M. M., Suzuki T., Sato I., Takewaki T., Suzuki K., Kobayashi H. (2005): Neuromechanical effects of pyrethroids, allethrin, cyhalothrin and deltamethrin on the cholinergic processes in rat brain. Life Sci. **77**, 795–807

Kalantzi O. I., Hewitt R., Ford K. J. (2004): Low dose induction of micronuclei by lindane. Carcinogenesis **25**, 613–622

Kaminski R. M., Tochman A. M., Dekundy A., Turski W. A., Czuczwar S. J. (2004): Ethosuximide and valproate display high efficacy against lindane-induced seizures in mice. Toxicol. Lett. **154**, 55–60

Katsumata K., Katsumata K. (2003): Norwegian scabies in an elderly patient who died after treatment with γBHC. Intern. Med. **42**, 367–369

Kwong T. C. (2002): Organophosphate pesticides: biochemistry and clinical toxicology. Ther. Drug Monit. **24**, 144–149

Martinez A. O., Martinez-Conde E. (1995): The neurotoxic effects of lindane at acute and subchronic dosages. Ecotoxicol. Environ. Saf. **30**, 101–105

Mićić D. V., Petronijević N. D. (2000): Acetylcholinesterase activity in the mongolian gerbil brain after acute poisoning with aluminium. J. Alzheimer Dis. **2**, 1–6

Mills P. K., Yang R. (2003): Prostate cancer risk in California farm workers. J. Occup. Environ. Med. **45**, 249–258

Mladenović D., Hrnčić D., Radosavljević T., Vučević D., Djurić D., Rašić-Marković A., Macut Dj., Šušić V., Šćepanović Lj., Stanojlović O. (2008): Dose-dependent anticonvulsive effect of ethanol on lindane-induced seizures in rats. Can. J. Physiol. Pharmacol. **86**, 148–152

Moreno M. J., Pellicer S., Fernández-Otero M. P. (1996): Effects of in situ and systemic lindane treatment on in vivo absorption of galactose and leucine in rat jejunum. Arch. Toxicol. **70**, 767–772

Muscat J., Britton J., Djordjevic M. (2003): Adipose concentrations of organochlorine compounds and breast cancer recurrence in Long Island, New York. Cancer Epidemiol. Biomarkers Prev. **12**, 1474–1478

Nagaraja T. N., Desiraju T. (1994): Brain regional variations in the levels of biogenic amines, glutamate, GABA and glutamate decarboxylase activity in developing and adult rats exposed chronically to hexachlorocyclohexane. Biogenic Amines **10**, 141–149

Ntow W. J. (2001): Organochlorine pesticides in water, sediment, crops, and human fluids in a farming community in Ghana. Arch. Environ. Contam. Toxicol. **40**, 557–563

Parmar D., Yadav S., Dayal M., Johri A., Dhawan A., Seth P. K. (2003): Effect of lindane on hepatic and brain cytochrome P450s and influence of P450 modulation in lindane-induced neurotoxicity. Food Chem. Toxicol. **41**, 1077–1087

Perrier N. A., Salani M., Falasca C., Bon S., Augusti-Tocco G., Massoulié J. (2005): The readthrough variant of acetylcholinesterase remains very minor after heat shock, organophosphate inhibition and stress, in cell culture and in vivo. J. Neurochem. **94**, 629–638

Pitler T. A., Alger B. E. (1992): Cholinergic excitation of GABAergic interneurons in the rat hippocampal slices. J. Physiol. (London) **450**, 127–142

Prado M. A. M., Reis R. A. M., Prado V. F., De Mello M. C., Gomez M. V., De Mello F. G. (2002): Regulation of acetylcholine synthesis and storage. Neurochem. Int. **41**, 291–299

Radosavljević T., Mladenović D., Vučević D., Petrović J., Hrnčić D., Djurić D., Lončar-Stevanović H., Stanojlović O. (2008): Effect of acute lindane and alcohol intoxication on serum concentration of enzymes and fatty acids in rats. Food Chem. Toxicol. **46**, 1739–1743

Raizada R. B., Srivastava M. K., Sarin S. (1993): Impact of technical hexachlorocyclohexane (HCH) on biogenic amines and locomotor activity of rat. Natl. Acad. Sci. Lett. (India) **16**, 73–76

Rauch A. E., Kowalsky S. F., Lesar T. S. (1990): Lindane (Kwell)-induced aplastic anemia. Arch. Intern. Med. **150**, 2393–2395

Rivera S., Rosa R., Martinez E., Sunol C., Serrano M. T., Vendrell M., Rodriguez-Farre E., Sanfeliu C. (1998): Behavioral and monoaminergic changes after lindane exposure in developing rats. Neurotoxicol. Teratol. **20**, 155–160

Rivera S., Sanfeliu C., Sunol C. (1991): Regional effects on the cerebral concentration of noradrenaline, serotonin, and dopamine in suckling rats after a single dose of lindane. Toxicology **69**, 43–54

Sahaya K., Mahajan P., Mediratta P. K., Ahmed R. S., Sharma K. K. (2007): Reversal of lindane-induced impairment of step-down passive avoidance and oxidative stress by neurosteroids in rats. Toxicology **239**, 116–126

Sahoo A., Samanata L., Chainy G. B. N. (2000): Mediation of oxidative stress in HCH-induced neurotoxicity in rat. Arch. Environ. Contam. Toxicol. **39**, 7–12

Soderlund D. M., Clark J. M., Sheets L. P., Mullin L. S., Piccirillo V. J., Sargent D., Stevens J. T., Weiner M. L. (2002): Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. Toxicology **171**, 3–59

Srivastava M. K., Raizada R. B. (2000): A limited three-generation reproduction study on hexachlorocyclohexane (HCH) in rats. Food Chem. Toxicol. **38**, 195–201

Trevisan R., Uliano-Silva M., Pandolfo P., Franco J. L., Brocardo P. S., Santos A. R. S., Farina M., Rodrigues A. L. S., Takahashi R. N., Dafre A. L. (2008): Antioxidant and acetylcholinesterase response to repeated malathion exposure in rat cerebral cortex and hippocampus. Basic Clin. Pharmacol. Toxicol. **102**, 365–369

Vučević D., Hrnčić D., Radosavljević T., Mladenović D., Rašić-Marković A., Lončar-Stevanović H., Djurić D., Macut Dj., Šušić V., Stanojlović O. (2008): Correlation between electrocorticographic and motor phenomena in lindane-induced experimental epilepsy in rats. Can. J. Physiol. Pharmacol. **86**, 173–179

Whittaker V. P., Barker L. A. (1972): The subcellular fractionation of brain tissue with special reference to the preparation of synaptosomes and their component organelles. In: Methods in Neurochemistry. (Ed. R. Fried), Marcel Dekker, New York