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Involvement of oxidative stress in liver injury after subchronic intoxication with low doses of chlorpyrifos – study on rats

Research Article

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Abstract: Organophosphate compounds are nowadays the most frequently used pesticides. For these insecticides, the primary target is acetylcholinesterase and for this reason the main clinical effect of acute intoxication with organophosphate insecticides involves an irreversible inhibition of the activity of this enzyme. However, in the chronic or subchronic exposition oxidative stress has been reported as the main mechanism of its toxicity. The present study investigated the effect of three low doses (0.2, 2, 5 mg/kg bw) of chlorpyrifos for 14 or 28 days on serum liver enzymes and on oxidative stress parameters in the liver of rats. Chlorpyrifos treatment resulted in aminotransferases and alkaline phosphatase increase after 14 days (higher doses) and 28 days (all doses) treatment together with changes of antioxidative enzymes activities and reduced glutathione and malonyldialdehyde level in the liver. The enhancement of lipid peroxidation is temporary, reaching a peak after 14 days and decreasing after 28 days of treatment. Based on the experimental findings of this study the temporary liver injury caused by oxidative stress has been shown. The disturbances in the liver antioxidative status and increased liver membrane permeability may appear in case of doses near to the accepted human daily intake.

Keywords: *Liver • Chlorpyrifos • Marker enzymes • Oxidative stress*

1. Introduction

Pesticides are a group of different chemicals, which has been developed to control a variety of pests. Organophosphate compounds are nowadays the most frequently used pesticides. The extensive use of organophosphates (OP) insecticides poses a risk to the people involved in its production and use in agriculture or households as well as to the population exposed to these compounds by consumption of the contaminated food products or water [1-3].

The mode of exposure to organophosphate insecticides includes the gastrointestinal, inhalatory and dermal ones. Poisoning, specially the acute one, occurs as a result of agricultural use, suicide or accidental exposure [1,4,5]. For these insecticides the primary target is acetylcholinesterase (AChE) that hydrolizes acetylcholine, a major neurotransmiter. For this reason the main clinical effect of acute intoxication with organophosphate insecticides involves an irreversible inhibition of the activity of this enzyme in the blood and in the nervous system resulting in the accumulation of acetylcholine and the activation of muscarinic and nicotinic receptors [1,4-6].

However, in the chronic or subchronic OP exposure the oxidative stress has been reported as the main mechanism of its toxicity [1,7]. It has been indicated that a lipid peroxidation in the majority of organs increases and that the enzymes associated with the antioxidant defense mechanism are altered under the influence of OP [1,8-10].

There are two main systems to counteract the damage caused by the reactive oxygen species (ROS) in the human and animal organism. One of them is the

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enzymatic system which consists of such enzymes as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) [1,11-14]. Another antioxidative system is non-enzymatic and it consists of a reduced form of glutathione (GSH) and vitamins: C, E and A. These two systems work synergistically to enhance the antioxidant capacity of the organism [1,11,12].

According to many authors, oxidative stress is induced in the acute and chronic intoxication with organophosphate compounds in the humans and experimental animals, which is manifested by changes in the activity of antioxidative enzymes and/or altered levels of non-enzymatic antioxidants in various organs of the organism (among them in liver). These authors have also shown the effects of oxidative stress in the form of increased concentration of e.g. malonyldialdehyde and carbonyl groups [1,7,9,15,16].

Liver is the key organ of metabolism, excretion and also detoxification of many xenobiotics, among them the therapeutic agents, and environmental pollutants [17-19]. Hence this organ is subjected to a variety of disorders.

The aim of present study was to investigate the liver disorder by monitoring changes in the serum activity of liver marker enzymes as well as the activity of liver antioxidative enzymes and concentration of GSH and malonyldialdehyde (MDA), being a lipid peroxidation marker as the indicators of oxidative stress after subchronic intoxication with low doses chlorpyrifos – one of the most commonly used OP insecticides.

2. Material and methods

2.1. Animals

Male Wistar rats (from the certificated Laboratory Animal House, Brwinow, Poland), 200–230 g body weight, were housed in the metal cages with a free access to drinking water and standard pellet diet.

2.2. Treatment and tissues collection

The animals received once daily, intragastrically by a stomach tube, 0.1 ml/100 g of olive oil (the control groups) and oil solution of chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridinyl phosphorothionate) at doses of 0.2, 2 or 5 mg/kg bw (the experimental groups). The chlorpyrifos-experimental groups or olive oil-control groups was administered for 14 or 28 days.

Chlorpyrifos (the certificated analytical standard; the purity value: min. 99.8% (m/m) was obtained from the Institute of Organic Industrial Chemistry, Warsaw, Poland. Chlorpyrifos was diluted with olive oil to the required concentration before the treatment.

The doses used in this experiment were far below the LD_{50} for chlorpyrifos (CPF) and caused no signs of cholinergic toxicity. The LD_{50} for chlorpyrifos was established at 95 mg/kg bw [20,21]. 24 h after the last insecticide or oil administration the rats were killed under anaesthesia with Vetbutal (pentobarbital sodium) and slices of livers were removed, washed in ice-cold 0.9% NaCl solution containing 0.16 mg/ml heparin and kept frozen at -70^{0}C until homogenized.

The blood was sampled by a direct heart puncture. Serum separation was performed by centrifugation of blood samples for 10 min., 3000 x g.

The livers were homogenized in potassium phosphate buffer (50 mM, pH 7.4) [22]. The homogenate was divided into three portions. The ice-cold metaphoshate acid solution was added to one portion of the homogenate and centrifuged at 3000 x g for 10 min. The assay of reduced glutathione (GSH) concentration was performed in a clear supernatant.

The second portion of homogenate was centrifuged at 700 x g for 20 min to determine the levels of malonyldialdehyde. The next portion of homogenate was centrifuged at 8500 x g for 30 min to determine (in supernatant) the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase.

The experimental procedure was approved by the Local Ethics Committee at Medical University in Bialystok. The experimental procedures were also performed in accordance with guidelines of Directive 86/609/EEC "European Convention for the Protection of Vertebrate Animals Used for Experimental and Others Scientific Purposes" (1986).

2.3. Biochemical estimation

The activity of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated by the commercially available kits BioMaxima, Poland. Serum alkaline phosphatase (ALP) activity was measured by BioSystems S.A. kit, Spain. The activity of serum liver enzymes was performed, according to the proper kit's instructions.

Determination of SOD, GPx and GR activity as well as GSH concentration was performed, according to the kit's instructions, using BIOXYTECH SOD-525™ Assay kit, BIOXYTECH GPx-340™ Assay kit, GR-340™ Assay kit and BIOXYTECH GSH-400™ Assay kit was produced by OXIS International, Inc., Portland, USA.

The SOD-525™ method is based on the SOD mediated increase in the rate of autoxidation of

5,6,6a,11b-tetrahydrobenzo[c]fluorene (R1) in aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm. Change in absorbance was measured after the addition of R1. The SOD activity was determined from the ratio of the autoxidation rates in the presence and in the absence of SOD. The GPx-340™ assay is an indirect measure of the activity of GPx. To assay the activity of this enzyme, brain homogenate was added to a solution containing glutathione, glutathione reductase and NADPH (nicotinamide adenine dinucleotide phosphatase-oxidase). The enzyme reaction was initiated by adding the substrate, tert-butyl hydroperoxide, and absorbance was recorded. The rate of decreases in absorbance was directly proportional to the GPx activity in the sample. The GR-340 assay is based on the oxidation of NADPH to $NADP^+$ catalyzed by a limiting concentration on glutathione reductase. One GR activity unit is defined as the amount of enzyme catalyzing the reduction of one mikromole of GSSG (glutathione disulfide) per minute at pH 6.6 and 25 ^{0}C.

The liver MDA concentration was measured with a BIOXYTECH MDA-586™ Assay kit according to the instruction. This method is designed to assay free MDA and is based on reaction of the chromogenic reagent, N-methyl-2-phelindole, with malondialdehyde at the temperature of 45^{0}C.

The liver CAT activity was determined according to the method described by Aebi [23]. It is based on the decomposition of H_2O_2 by catalase. The reaction mixture was composed of 50 mM phosphate buffer of pH 7.0; 10 mM H_2O_2 and liver homogenate. The reduction rate of H_2O_2 was followed at 240 nm for 30 sec. at room temperature.

The enzymatic activities (SOD, CAT, GPx and GR) were expressed as units of the enzymatic activity per milligram or gram of protein. The protein levels were determined after dilution of proper supernatant by the Lowry method [24]. The GSH concentration was expressed as μmol of GSH per gram of tissues. The concentration of MDA was expressed as nmol MDA per gram of tissue.

2.4. Statistical analysis

The data are expressed as mean±standard derivation (SD) of 9 observations. Differences among experimental groups were determined by one-way ANOVA. The comparison between the means was carried out using the Tukey-Kramer multiple comparison tests. In all experiments, P-values lower than 0.05 were considered to be a statistically significant. Spearman correlations between the study parameters were calculated.

3. Results

A significant increase, when compared to the control groups, in the activities of serum ALT, AST and ALP was observed after 14 days (except intoxication with lowest insecticide dose) and 28 days of treatment with chlorpyrifos (Table 1).

The increase in the serum activity of ALT and AST was found to be dose dependent after 14 days of treatment. There are no statistically significant differences after 28 days of chlorpyrifos treatment on the rats at doses of 0.2 and 2 mg/kg. The highest increase in the AST and ALP activity in the serum was found after 28 days of the insecticide intoxication. ALT activity stayed at the same level after 14 and 28 days of the treatment.

The enhancement of serum ALT activity was more pronounced after chlorpyrifos administration than serum AST activity. The increases in ALT activities in serum were 0%, 38%, and 127% after 14 days of exposure and 50%, 73%, and 133% after 28 days of exposure with

Table 1. Activity of liver enzymes in serum after chlorpyrifos intoxication

Days of treatment	Dose of CPF mg/kg/day	ALT (U/l)	AST (U/l)	ALP (U/l)
14	0	20.68±1.53	45.13±4.83	55.36±3.17
	0,2	21.84±2.30	48.57±1.86	55.58±3.49
	2	28.54±4.70^{ab}	54.11±5.84a	84.66±3.68^{ab}
	5	46.95±4.62^{abc}	65.82±3.67^{abc}	88.65±2.84^{ab}
28	0	19.37±1.77	44.03±1.94	56.67±2.31
	0,2	29.17±3.26^{ad}	50.47±3.84^{a}	63.16±4.32^{ad}
	2	33.50±5.25^{ad}	54.67±6.55^{a}	67.03±4.18^{ad}
	5	45.16±4.86^{abc}	71.14±3.54^{abcd}	120.14±7.13^{abcd}

Values are expressed as means±SD (n = 9)
$p < 0.05$ in relation to:
a – control groups
b – group intoxicated with chlorpyrifos at dose 0.2 mg/kg bw/day
c – group intoxicated with chlorpyrifos at dose 2 mg/kg bw/day
d – group after 14 days of intoxication

chlorpyrifos but the increases in AST activities were 0%, 19%, 45% and 14%, 24%, 61% respectively.

GSH level was found to increase (by 140% and 14% respectively) in the liver of rats after the chlorpyrifos administration at doses of 0.2 and 2 mg/kg/ bw and to decrease by 33% after the insecticide administration at a dose of 5 mg. After 28 days of lower doses intoxication the liver GSH level decreased in comparison to the shorter period of treatment but was still (at a dose of 0.2 mg/kg bw/day) above the control value. The increase (in comparison to the shorter period) in this parameter level was observed in the liver of rats after administration of 5 mg/kg/ bw of chlorpyrifos. In this group of rats the GSH level was still below the control value (Table 2).

GPx activity was higher in comparison to the control value after 14 days of insecticide administration whereas after 28 days of daily treatment at a dose of 5 mg, GPx activity in the liver diminished in comparison to the control value (Table 2).

CPF exposure for 14 and 28 days caused an increase in the liver GR activity (Table 2). The highest increase in this enzyme activity was observed in the liver of rats receiving chlorpyrifos at dose 5 mg daily for 28 days. In this group of rats the liver GR activity was higher in comparison to shorter period of intoxication with the same dose and in a comparison to the groups of rats receiving lower insecticide doses (Table 2).

The chlorpyrifos treatment caused an increase in the liver SOD activity after 14 days of exposure as well as after 28 days. However, an increase in the liver CAT activity was observed only after 14 days of treatment with the lowest dose and after 28 days of treatment with all examined doses (Table 3).

MDA level was found to increase in the liver of rats treated with chlorpyrifos at all examined doses for 14 days and 28 days. The highest MDA level was observed after 14 days of treatment with 5 mg/kg bw of chlorpyrifos. The MDA level for this group of rats was higher by 180% in comparison to the control group and by 146% and 14% in comparison to the groups of lower doses intoxication (Table 3).

Table 2. Reduced glutathione level and activities of glutathione related enzymes in liver of rats after chlorpyrifos intoxication

Days of treatment	Dose of CPF mg/kg/day	GSH (mmol/g tissue)	GPx (U/g protein)	GR ((U/g protein)
14	0	275.52±26.69	186.44±32.81	31.64±1.23
	0,2	661.79±111.77[a]	247.24±21.45[a]	34.67±1.81[a]
	2	313.58±34.03[ab]	228.73±22.73[a]	35.84±2.92[a]
	5	183.70±24.44[abc]	262.46±18.07[ac]	36.46±2.59[a]
28	0	269.84±14.60	190.10±5.68	30.91±1.75
	0,2	282.67±8.95[ad]	194.83±25.77[d]	37.84±5.48[a]
	2	262.36±17.84[bd]	184.79±14.72[d]	42.36±3.71[ad]
	5	238.51±11.71[abcd]	169.88±19.60[ad]	53.31±4.44[abcd]

Values are expressed as means±SD (n = 9)
p < 0.05 in relation to:
a – control groups
b – group intoxicated with chlorpyrifos at dose 0.2 mg/kg bw/day
c – group intoxicated with chlorpyrifos at dose 2 mg/kg bw/day
d – group after 14 days of intoxication

Table 3. Activities of antioxidative enzymes and malonyldialdehyde concentration in liver of rats after chlorpyrifos intoxication

Days of treatment	Dose of CPF mg/kg/day	SOD (U/mg protein)	CAT (U/mg protein)	MDA (nmol/g tissue)
14	0	199.75±4.47	109.59±10.53	48.49±3.28
	0,2	245.77±28.14[a]	142.29±19.35[a]	55.10±3.80[a]
	2	273.93±8.82[a]	120.34±22.17[b]	119.10±19.26[ab]
	5	327.70±27.68[abc]	117.23±13.24[b]	135.65±9.26[ab]
	0	201.85±2.84	110.55±8.21	50.89±2.52
28	0,2	219.68±15.19[ad]	171.01±22.87[ad]	61.89±8.07[a]
	2	280.69±7.88[ab]	181.71±12.54[ad]	73.68±2.94[abd]
	5	311.70±39.58[abc]	219.70±25.73[abcd]	83.01±4.38[abcd]

Values are expressed as means±SD (n = 9)
p < 0.05 in relation to:
a – control groups
b – group intoxicated with chlorpyrifos at dose 0.2 mg/kg bw/day
c – group intoxicated with chlorpyrifos at dose 2 mg/kg bw/day
d – group after 14 days of intoxication

After 28 days of intoxication with doses of 2 and 5 mg/kg bw/day, the liver MDA level decreased by 38% and 26% respectively in comparison to the group of rats intoxicated with these doses for 14 days (Table 3). The values of MDA level after 28 days of treatment were still statistically significant higher in comparison to the control group.

A positive correlation was found between MDA and ALT (r = 0.781), MDA and AST (r = 0.698) and MDA and ALP (r = 0.786) while negative correlation was found between liver GSH level and activity of serum ALT (r =–0.603), AST (r =–0.519) and ALP (r =–0.444). The positive correlation was found between SOD and MDA (r = 0.783). All these correlations were statistically significant ($p<0.05$).

4. Discussion

Chlorpyrifos – insecticide used in this work, is moderately toxic to rats [21]. For humans, an accepted daily intake was established on the level of 0-0.01 mg/kg bw [2]. This was on the basis of a NOAEL (no observed adverse effect level) of 1 mg/kg bw per day for an inhibition of the brain acetylcholinesterase activity in the study on rats, using a 100-fold safety factor, and on a NOAEL of 0.1 mg/kg bw per day for an inhibition of the erythrocyte acetylcholinesterase activity in the study of human subjects exposed for nine days, using a 10-fold safety factor [25]. It is important to mention that the lowest dose used in this study is only two times higher than NOAEL for humans.

According to many authors, oxidative stress is induced in both acute and chronic intoxication with the organophosphate compounds in humans as well as in the experimental animals and is manifested by the changes in the activity of antioxidative enzymes and/or the changed levels of non-enzymatic antioxidants and enhancement of MDA level – lipid peroxidation marker [9,15,26-28]. Reactive oxygen species are hazardous to human and animal health and causes degenerative effects on different organs [1,18,22].

The liver is especially prone to the xenobiotics induced injury because of its central role in the xenobiotics metabolism, its portal location within the circulation, and its anatomic and physiologic structure [17,18]. The measurement of various enzyme activities in the serum provides a significant and well known aid in investigation of the organ injury. The nature of enzymes released from damaged tissue depends on its subcellular localization [16-18]. The serum aminotransferases activities, including AST and ALT, are commonly referred to as “liver enzymes,” because they are abundantly present within hepatocytes and these enzymes are released from the damaged hepatocytes into the blood. For this reason their activities measured in the serum have been widely used to detect a liver injury [29].

Another enzyme which activity has been examined in this study – serum alkaline phosphatase, increases to some extent in the most types of liver injuries but the highest serum activity of this enzyme is observed in the cholestatic injuries [18].

The present study has shown that serum ALT, AST and ALP activities increased in comparison to the control value after the chlorpyrifos treatment with doses of 2 and 5 mg/kg bw daily for 14 days and after the treatment for 28 days with doses of 0.2, 2 and 5 mg/kg bw daily. This increase occurred much more as a parallel to the dose, especially for ALT and AST activities after the shorter period of intoxication. chlorpyrifos treatment caused higher increase ALT activity in the serum than AST activity.

ALT and AST are important indicators of a liver injury. The higher specific for liver has ALT, whereas AST is also located in the heart, brain, kidney, and skeletal muscle, what makes this enzyme less specific [17,18,30]. The increase in serum activities of these enzymes is a result of cellular membrane damage and leakage. Their serum level depends on the relative rate at which they enter and leave the circulation [17,18,30]. Most liver diseases and injuries are characterized by greater ALT than AST elevations.

Increased serum liver enzyme activity (ALT, AST and ALP) was accompanied by the changes of antioxidative enzymes (SOD, CAT, GPx and GR) as well as GSH and MDA concentration in the liver. These effects were generally observed in treatment doses of 0.2, 2 and 5 mg/kg bw daily, for 14 or 28 days but there were not dose-dependent.

An increase in the activity of liver enzymes in the serum and elevated liver MDA level of rats subchronically intoxicated with chlorpyrifos confirms that a functional integrity of liver membrane has been compromised and suggest that this increased liver membrane permeability is due to the enhancement of lipid membrane peroxidation. Also, a positive (highly significant) correlation between ALT, AST, ALP and MDA confirms this supposition. In general, after subchronic chlorpyrifos treatment, ALT, AST and ALP showed a similar profile to MDA level. Thus observed changes confirm the involvement of oxidative stress in liver injury after the subchronic low doses chlorpyrifos intoxication of rats.

Oxidative stress is a balance between free radical production and antioxidant activity. It is possible that the raised MDA level may be due to a decreased antioxidant activity [1]. In the present study changes in the activity of antioxidant enzymes as well as in GSH and MDA concentration in the liver of chlorpyrifos treated rats have been observed. Suprisingly, prolonged chlorpyrifos treatment (for 28 days) caused a marked decrease in MDA level at the higher doses and this effect for highest dose may be regarded as recovery effect by replenishment of GSH, as indicated by significant increase of this parameter, in comparison to shorter duration of intoxication.

It is well known that in a defense against oxidative stress GSH has an important function. As reported by other authors, the glutathione protects liver in many different ways: it can directly remove ROS or it can act as a substrate for GPx and glutathione-S-transferase (GST) – enzyme which takes a part in detoxifing of xenobiotics or their metabolites. GSH reduced a large amount of hydroperoxides and in this way it prevents unsaturated lipids from oxidation [1,13,14,31].

It has been emphasized that in the liver of rats subchronically intoxicated with chlorpyrifos a change of GSH level depends on the insecticide dose and duration of treatment. The GSH level after 14 days intoxication reached 363% of the control value for a dose of 0.2 mg/kg bw, 114% and 67% of control values for the higher doses but after 28 days treatment GSH level decreased for doses of 0.2 and 2 mg/kg bw and increased for a dose of 5 mg/kg bw daily (in comparison to shorter duration of intoxication). The increased level of GSH after the lowest dose intoxication is a defense mechanism against oxidative stress induction by chlorpyrifos whereas the decreased level after the highest dose intoxication probably is due to the rate of ROS generation which is an overwhelming possibility of GSH regeneration. The decrease in liver GSH level after 14 days highest dose chlorpyrifos treatment reflects the increased lipid peroxidation index.

The activities of glutathione related enzymes – GPx and GR- did not change in a dose-dependent manner. The activity of GPx increased after 14 days of chlorpyrifos treatment (the highest value was for the group of 5 mg/kg bw per day treatment) in comparison to the control value and decreased after 28 days insecticide treatment, in comparison to the value received after a shorter period of intoxication and for the highest dose also in a comparison to the control value. An increased activity of GPx may be due to the significant production of H_2O_2 in the pesticide-induced toxicity [1]. GPx uses GSH in coping with ROS and in this reaction the level of reduced glutathione is diminished [1]. The decreased level of GSH accompanied by increase in GPx activity in group of rats treated with highest dose of chlorpyrifos for 14 days are in accordance with these findings.

Chlorpyrifos intoxication resulted in an increased liver GR activity, in comparison to the control value. The elevated GR activity increased the GSH level because glutathione is maintained in the reduced state, as it is known, by GR. In the present study, after 28 days of treatment an increase in the liver GR activity was higher than in the shorter period of chlorpyrifos administration. The highest value of GR activity has been observed after 28 days of chlorpyrifos treatment with a dose of 5 mg/kg bw daily and this enzymatic increase probably leads to restoration of GSH level as indicated by change of its level.

The higher rate of change in liver GSH level than glutathione related enzymes after chlorpyrifos treatment may be associated glutathione involvement in non-enzymatic reaction with reactive oxygen species, or its participation in the conjugation reactions – GSH acts as a substrate for GST [1,13,14,31]. The elevated GST activity and reduction in GSH levels in the liver have been reported by Rae [32], after the subchronic intoxication with OP insecticide in fish. GST-mediated conjugation may be an important mechanism for detoxifying peroxidised lipid breakdown products [32].

The subchronic intoxication with chlorpyrifos caused an increase in the liver SOD activity after 14 and 28 days of treatment. Activity of this enzyme did not change in a dose or time related manner, except the highest dose, where SOD activity after 14 and 28 days exposition was statistically significant higher in comparison to the lower doses intoxication. The increase in superoxide dismutase activity in the liver of chlorpyrifos exposed rats appears to be due to the intensified generation of reactive oxygen species. This observation has been confirmed by a positive correlation between SOD activity and MDA level. Thus data of the present work suggests that the chlorpyrifos treatment results in an increased formation of reactive oxygen species, which stimulates an increase in the liver SOD activity to cope with oxidative stress.

SOD is an ‘‘incomplete antioxidant’’, which scavenges superoxide anion radical and contributes to overproduction of hydrogen peroxide – a more reactive particle then superoxide anion [10]. However in the present work, despite of the increased activity of SOD, the liver CAT activity – hydrogen peroxide removing enzyme stayed on the control level (except for the lowest dose) after 14 days and it increased after 28 days of the chlorpyrifos treatment. There is no correlation between the activities of CAT or GPx and MDA concentration.

In the view of present study, the results seem to be clear that a nature of chlorpyrifos-induced alterations in the liver antioxidative enzymes activity and glutathione concentration as well as MDA level is determined by the dose and the duration of the exposure. However, it is interesting to note that the chlorpyrifos kinetic studies indicate that enhancement of lipid peroxidation is temporary, reaching a peak after 14 days and decreasing after 28 days of treatment.

The experimental findings of the current study suggest that the consequences of oxidative stress in liver depend mostly on GSH level. The increased level of GSH after 14 days of 0.2 mg/kg bw chlorpyrifos treatment leads to a slight but statistically significant increase in MDA level and unchanged activity of ALT, AST and ALP in the serum. An increased GSH level leads to a decrease in MDA concentration as it has been shown in the group of rats after 28 days of treatment with chlorpyrifos doses of 2 and 5 mg/kg/ bw. Thus the increased level of GSH is associated with a decreased lipid peroxidation index in the chlorpyrifos treated rats.

In conclusion, the present study has shown that low doses of chlorpyrifos treatment caused liver injury and this injury occurred by free radical mechanism. Fortunately, the change in liver marker enzymes as well as examined antioxidative parameters and lipid peroxidation index in liver seems not to be persistent. In subchronic chlorpyrifos intoxication an activation of the compensatory to oxidative stress mechanism is involved what leads to a reversible liver injury.

The disturbances in the liver antioxidative status and the increased liver membrane permeability might appear in doses near the accepted human daily intake.

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