Journal of Environmental Protection and Ecology 11, No 1, 247–252 (2010)

TOXIC EFFECT OF SOME DANGEROUS CHEMICALS ON SUPEROXIDE DISMUTASE ENZYMATIC ACTIVITY

S. IVAN*, I. LUCACIU, G. RUSU, I. VASILE

National Research and Development Institute for Industrial Ecology – ECOIND, 90–92 Panduri Avenue , 050 663 Bucharest – 5, Romania
E-mail: ecoind@incdecoind.ro; www.incdecoind.ro

Abstract. The research work presents the evaluation of superoxide dismutase enzymatic activity modifications as a result of toxic effect of dangerous chemicals. Superoxide dismutase is an efficient enzymatic antioxidant and plays an important role in the protection reaction of organism against negative effects of the free radicals. Therefore it is necessary to study this enzyme for determination of dangerous chemicals toxicity on it. For this study two pesticides were selected: atrasine and mevinphos, whose toxicity characteristics have been determined after performing ecotoxicological test to evaluate their risk on aquatic organisms (fish of *Cyprinus carpio* species), sensitive to the toxic action of compounds taken in study. In order to obtain relevant results, an enzymatic kit for measure of superoxide dismutases (MnSOD and CuZnSOD) has been used. Measurements were performed from protein extracts obtained from intoxicated fish organs. The biochemical method is a spectrometrical method and tetrazolium salt is used for superoxide radical detection, produced by xantinoxidase. The obtained results have led to the conclusion that dangerous chemicals taken in study determine catalytic activity modifications of superoxide dismutase toward blank. In addition, in case of mevinphos the enzyme may be completely inhibited, this being suffocated by the proper metabolites (H_2O_2 or *OH). These modifications of superoxide dismutase activity can lead to drastic consequences on aquatic organisms.

Keywords: superoxide dismutase, toxicity, dangerous chemicals.

AIMS AND BACKGROUND

The research paper has in view to estimate the adverse toxic effect induced by dangerous chemicals from the class of pesticides (herbicides – atrazine and insecticides – mevinphos), specified in the Governmental Decision No 351/21.04.2005, on aquatic organisms.

The toxic effect of these chemicals is measured through antioxidant system modifications and especially by superoxide dismutase enzymatic activity changes. Superoxide dismutase (SOD) is an efficient enzymatic antioxidant and plays an important role in the protection reaction of organism against negative effects of the free radicals.

* For correspondence.

Together natural antioxidants represented by superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes act in detoxification process against free radicals that may induce oxidative stress and as result the death of organisms. Figure 1 shows the antioxidant reactions complexity where superoxide dismutase plays a crucial role in H_2O_2 transformation.

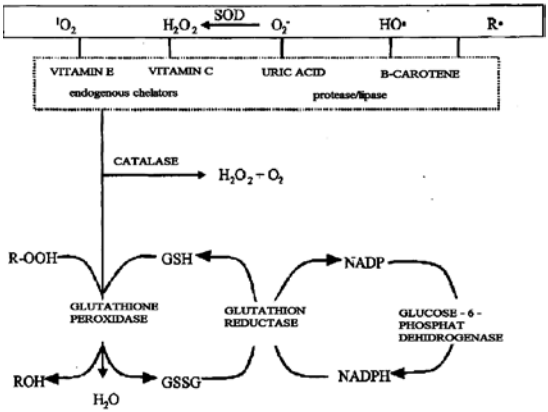


Fig. 1. Antioxidant system reactions (SOD – superoxide dismutase; R–OH – alcohol; R–OOH – peroxides; GSH – reduced glutathione; GSSG – oxidised glutathione; NADP – nicodinamine adenine dinucleotide phosphate; NADPH – reduced form of nicodinamine adenine dinucleotide phosphate)

Scientific works revealed that atrazine and mevinphos may induce oxidative stress with direct and even devastating effects especially on the cell structure and function, cellular apoptosis and the death of the intoxicated organisms[1,2]. Superoxide dismutase may be considered a bioindicator of this complication that occurs in case of contamination[3].

This work evaluates the toxic potential of studied chemicals on aquatic organisms, e.g. fish, as well as their noxious effect manifested on antioxidant enzymes activity (represented by superoxide dismutase) of liver, gill, white muscle tissue and intestine, sampling from viable juvenile carp (*Cyprinus carpio* sp.*)*, surviving after the acute and chronic toxicity biotests performing.

EXPERIMENTAL

BIOLOGICAL MATERIAL

The aquatic organisms utilised in acute toxicity biotests and chronic toxicity studies were represented by 1-year old juvenile carp with health and origin certificate, taken from selected lots of the Research and Development Centre for Pisciculture NUCET – Dambovita district, from experimental tanks populated with juvenile fish from healthy animals. After acquisition, the fishes with similar length, weight

and age, these were acclimatised in laboratory conditions, in maintenance aquaria from aquatic biobasis of INCD-ECOIND (Ref. 4).

The biological material, utilised in biochemical analysis, was subjected to acute toxicity biotests (tested solutions: 50, 25, 10 and 1 mg/l of atrazine and 10, 1, 0.1, 0.01 mg/l of mevinphos) and chronic toxicity studies (tested solutions: atrazine 0.01 mg/l and mevinphos 0.001 mg/l). The biochemical analysis was performed on viable fish resulted after acute intoxication with 10 and 1 mg/l atrazine, 0.1 and 0.01 mg/l mevinphos; and chronic intoxication with 0.01 mg/l atrazine and 0.001 mg/l mevinphos, for the rest of concentrations 100% mortalities were registered.

SAMPLING/PREPARATION OF PROTEIN EXTRACTS

After sampling, interest organs (liver, gill, intestine and white muscle tissue) were kept on ice and were subject to the next treatment with a view to preparation of protein extracts: individual washing with phosphate buffer 7.4 pH; mechanic disintegration of organs through homogenisation with quart sand; homogenisation with 20 mM cold HEPES buffer, 7.2 pH, containing 1 mM EDTA, 210 mM manitol and 70 mM sucrose, per g tissue; 10 min centrifugation at 4500 rpm/min and separation of supernatant with enzymatic activity (protein extract).

BIOCHEMICAL METHODS

The Lowry method for determination of total protein concentration from protein extracts is based on cupric complex formation when the protein reacts with a cuprum alkaline reagent and on reduction of phosphomolybdate and phosphowolframate by protein phenolic compounds of the Folin-Ciocalteu reagent.

Calbiochem method for determination of superoxide dismutaze enzymatic activity (the Merck enzymatic kit) is based on SOD spectrophotometer determination (at 450 nm) through tetrazolium salt utilisation for superoxide radicals detection generated by xantineoxidase. The work procedures implicate curve calibration, according with Table 1, obtained for final superoxide dismutase enzymatic activity determination (Fig. 2).

Table 1. Superoxide dismutase calibration solutions

No tube	SOD stock (µl)	Sample buffer (µl)	Final enzymatic activity (U/ml)
A	0	1000	0
B	20	980	0.025
C	40	960	0.05
D	80	920	0.1
E	120	880	0.15
F	160	840	0.2
G	200	800	0.25

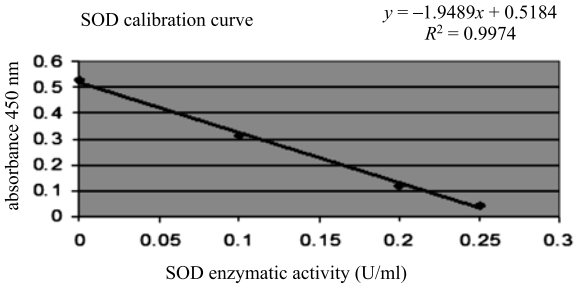


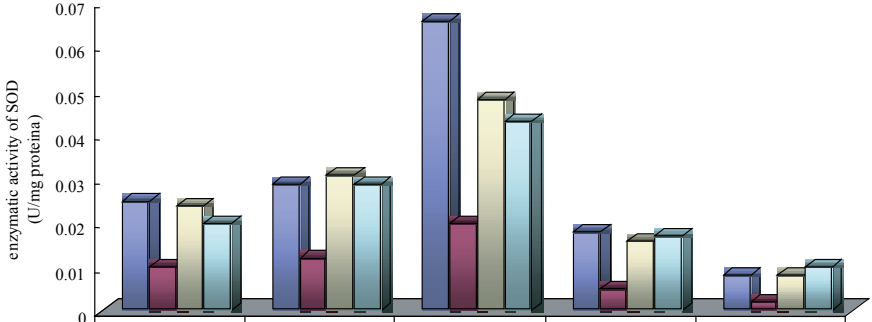
Fig. 2. Calibration curve for superoxide dismutase activity

RESULTS AND DISCUSSION

The health of aquatic organisms was evaluated from the point of view both of biochemical and physiological modifications. Before and after dissection, the fish that survived after toxicity biotests was visually investigated, and the next modifications were observed: in case of acute intoxication – equilibrium lost, tegument depigmentation, bile and liver increase in volume; and, in case of chronic intoxication, the abnormal physiological modifications were not observed.

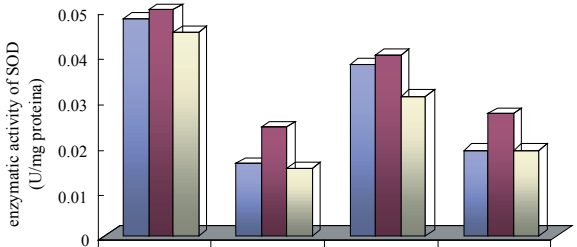
The enzymatic activity of superoxide dismutase was detected in all analysed organs but particularly in the liver (0.025±0.01 U/mg) and tissue level (0.024±0.001 U/mg).

- *as a result of the acute toxicity biotest*, the atrazine has determined an increase of superoxide dismutase activity specially in liver (Fig. 3) obtaining a value of 0.066÷0.01 U/mg (P<0.001), while at the muscle tissue level was obtained value of 0.020±0.02 (P<0.001); and the mevinphos inhibits significantly superoxide dismutase activity, the most affected organ again was the liver – 0.008 ±0.001 U/mg (P<0.001) – followed by the muscle tissue and the intestine. The toxic effect manifested by the mevinphos can be explained by the excess of free radicals resulted after metabolic reactions in which this antioxidant enzyme can not completely cope with.
- *as a result of chronic toxicity studies*, the biochemical analysis of samples did not show toxic character of the tested concentrations (0.1 mg/l atrazine and 0.001 mg/l mevinphos) (Fig. 4).



	blank	atrazine 1 mg/l	atrazine 10 mg/l	mevinphos 0.01 mg/l	mevinphos 0.1 mg/l
liver	0.025	0.029	0.066	0.018	0.008
intestine	0.01	0.012	0.02	0.005	0.002
muscular tissue	0.024	0.031	0.048	0.016	0.008
gill	0.02	0.029	0.043	0.017	0.01

Fig. 3. Variation of superoxide dismutase activity in organs intoxicated with atrazine and mevinphos – CL_{50}



	liver	intestine	muscular tissue	gill
blank	0.048	0.016	0.038	0.019
atrazine 0.1 mg/l	0.05	0.024	0.04	0.027
mevinphos 0.001 mg/l	0.045	0.015	0.031	0.019

Fig. 4. Variation of superoxide dismutaze activity in organs intoxicated with atrazine and mevinphos – MATC

CONCLUSIONS

The antioxidants enzymes (represented by superoxide dismutase) are affected by different pollutions, which can affect the enzymatic activity at the level of different organs by their inhibition, comparatively with the control group[3].

The studied pesticides (atrazine and mevinphos) have determined changes in the activity of antioxidant system, the detoxifying function of superoxide dismutase in case of acute intoxication was discredited, resulting in high death rate during the test.

The obtained results after acute biotests led to the conclusion that the dangerous chemicals in this study determine the catalytic activity modifications of superoxide dismutase towards blanc. In addition in case of mevinphos the enzyme may be completely inhibited, being suffocated by the proper metabolites (H_2O_2 or *OH) (Ref. 5). These modifications of superoxide dismutase activity can lead to drastic consequences on aquatic organisms.

The studies of chronical toxicity and biochemical analysis of aquatic organisms showed that in the tested concentrations, both the atrazine (0.1 mg/l) and the mevinphos (0.001 mg/l) did not give adverse effects, the obtained values being between the limits determined for control. In this case, the antioxidant system takes part in the organism detoxification, the metabolisation of contaminants, eliminating free radicals that appeared because of intoxication and avoiding their accumulation in tissues and organs.

REFERENCES

1. M. O. JAMES, J. B. PRITCHARA: Pesticide Metabolism in Aquatic Organism. Research Triangle Park, NC 27709, USA.
2. LIU XIN MEI, SHAO, JIAN-ZHONG, XIANG, LI-XIN: Cytotoxic Effects and Apoptosis Induction of Atrazine in Grass Carp (*Ctenopharyngodon idellus*) Cell Line. Environmental Toxicology, **21** (1), 80 (2006).
3. J. H. JEE, J.-C. KANG: Biochemical Changes of Enzymatic Defense System after Phenanthrene Exposure in Olive Flounder *Paralichthys olivaceus*. Physiologic. Res., **54**, 585 (2005).
4. G. RUSU, I. LUCACIU, E. P. LEONTE: Ecotoxicological Studies to Evaluate the Risk on Environmental of Dangerous Chemicals (Toxicity Biotests). INCD – ECOIND, Bucharest, 2006.
5. M. COMPORTI, A. POMPELLA: Toxicological Significance of Free Radicals. In: Free Radicals in the Environmental, Medicine and Toxicology. Richelieu Press, London, 1944.

Received 5 March 2008
Revised 16 July 2008