Global Veterinaria 2 (5): 250-256, 2008
ISSN 1992-6197

Oxidative Stress in Rats Exposed to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin with Emphasis on the Magnetic Properties of Iron Atom in Hemoglobin

[1]M.R. Bassem and [2]A.S.M. Fouzy

[1]Department of Biochemistry,
Genetic Engineering and Biotechnology Division, National Research Center, Dokki, Giza, Egypt
[2]Department of Food Toxicology and Contaminants, National Research Center, Dokki, Giza, Egypt

Abstract: Dioxin is the name generally given to a class of super-toxic chemicals, formed as a by-product of the manufacture, molding, or burning of organic chemicals and plastics that contain chlorine. It is the nastiest, most toxic man-made organic chemical; its toxicity is second only to radioactive waste. Female rats of 4-6 weeks old were used in this experiment to study the oxidative stress of 2,3,7,8-tetrachlorodibenzo-p-dioxin and its effects on the magnetic properties of the iron atom in hemoglobin of rats. Rats were divided into two groups: G1 that contained 10 rats as control and G2 contained 15 rats received 0.25% of the lethal dose (LD_{50}) of Dioxins compounds (17 congeners) orally. Hemoglobin magnetic susceptibility, auto-oxidation rate, different hemoglobin derivatives concentration, antioxidants activity (SOD and glutathione peroxidase) and molar heme concentration were measured. Hemoglobin magnetic susceptibility in the group exposed to dioxin showed lesser negative value with elevated abnormal derivative concentrations. Also, higher auto-oxidation rate with increased anti-oxidant activity was recorded in dioxin intake group as compared to the control group. It was concluded that 2,3,7,8-tetrachlorobinzo-*p*-dioxin is highly toxic to the infrastructure of hemoglobin as it affects the hemoglobin magnetic properties by shifting the molar magnetic susceptibility to the ferromagnetic region as a result of abnormal hemoglobin derivatives formation. This may result in converting an amount of hemoglobin to forms unable to carry out normal functions of blood.

Key words: Dioxins · Hemoglobin · Oxidative stress · Magnetic properties

INTRODUCTION

The halogenated aromatic hydrocarbons are a group of ubiquitous, toxic environmental contaminations of which the chlorinated dibenzo-*p*-dioxins are considered to be the most potent compounds [1]. These groups of compounds have been shown to induce toxicity, primarily through interaction with the aryl hydrocarbon receptor (AHR), [2], a cytosolic protein belonging to the basic-helix superfamily of DNA-binding proteins [3]. Among the dibenzo-*p*-dioxins, 2,3,7,8-tetrachlorobinzo-*p*-dioxin (TCDD), commonly known as dioxin, exhibits the greatest toxicity, has been shown to cause a wide range of biochemical and pathological changes in mammalian and non-mammalian species [4].

Dioxin form as unwanted impurities in the manufacturing of chlorophenol and its derivatives. Also, it is produced during the combustion of municipal, sewage-sludge, hospital and hazardous wastes. Combustion, in presence of a chlorine donor, seems to be a major source of these compounds. High levels of dioxins are also emitted from metallurgical industries including copper smelters, electric furnace in steel mills and wire reclamation incinerators. Trace levels are detectable in emissions from motor vehicles using leaded gasoline or diesel fuel, in forest fires and in residential wood burning [5]. The elimination half life of 2,3,7,8-tetrachlorodibenzo-p-dioxin in humans is approximately 7-11 years [6].

TCDD produces a wide spectrum of toxic effects, most of its toxicity is due to oral exposure. Very few percutaneous and no inhalation exposure data are available in the literature. There is a wide range of difference in sensitivity to TCDD lethality in animals. The signs and symptoms of poisoning with chemicals contaminated with TCDD in humans are analogous to those observed in animals [7].

Corresponding Authors: Dr. M.R. Bassem, Genetic Engineering and Biotechnology Division, Biochemistry Department, National Research Center, Postal Code: 12622, Egypt E-mail: bassemraafat@hotmail.com

Dioxin are absorbed by passive diffusion. After being absorbed, these compounds are rapidly distributed to tissues with high lipid content. In most animals studied, the major site of dioxin retention is the liver. Broad species variations have been observed in metabolism and excretion [8].

The position that humans occupy regarding metabolic fate and excretion relative to other species is still undetermined. A healthy adult volunteer who ingested 1.14 ng kg^{-1} of H-TCDD in corn oil had almost complete absorption (>87%) through the gastrointestinal tract and 90% of the body burden of TCDD sequestered in fat [9]. Dioxin residues including TCDD have been found in adipose tissue, blood serum and breast milk of the general population from highly industrialized countries [10].

Oxidative stress can be viewed as the disturbance in the oxidant-antioxidant balance in favor of the former. It is well established that acute high-dose exposure to TCDD results in oxidative stress in multiple tissues and species [11]. Oxidative stress from TCDD exposure in laboratory animals increases the production of reactive oxygen species (ROS), lipid peroxidation and DNA damage [12, 13]. Oxidative stress following TCDD administration is aryl hydrocarbon (Ah) receptor-mediated [14]. A recent study demonstrated that TCDD causes a sustained oxidative stress response in female C57BL/6J mice that persists as long as 8 weeks following administration of 5 mg kg^{-1} on 3 successive days [15]. In addition, recent studies reported an elevation of oxidative stress markers in the brain following subchronic doses as low as 0.45 ng TCDD/kg/day [16]. However, oxidative stress resulting from low-dose subchronic TCDD exposure has not been fully characterized and a relationship between TCDD tissue concentration and the corresponding oxidative stress response has not been clearly defined.

One possible mechanism of TCDD-mediated ROS production has been proposed to involve the cytochrome P450s [17]. Due to their membership in the Ah gene battery, CYP1A1 and CYP1A2 have been suggested to be associated with TCDD-mediated oxidative stress [18]. One proposed pathway is through the metabolic activation of estrogen via TCDD-induced cytochrome P450 enzymes [19]. The involvement of cytochrome P450s in the production of ROS during normal enzyme function has long been known [20-22] and may also contribute to TCDD-induced ROS. Although water is a normal product of the electron transfer in the CYP450 catalytic cycle, hydrogen peroxide can be produced by specific isoforms in addition to other ROS. The physiologic consequence of this free radical formation has not been thoroughly investigated. The large induction of CYP1A1, 1A2 and 1B1 by TCDD may result in increased free radical formation.

This work was design to study the oxidative stress following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Special interest was given to the changes in the magnetic properties of the iron atom in hemoglobin of exposed rats.

MATERIALS AND METHODS

1-Experiment Animals: Female rats of 4-6 weeks old were obtained from the Animal House of the National Research Centre, animals were maintained for acclimatization before initiation of the experiment, pass through normal day and light periods, with prevailing temperature of 22±1°C, humidity 40-60% and allowed free access to food and water. The initial rat weight was approximately 100 g. These young rats were in a rapid growth phase and were selected to simulate the rapid growth phase seen in human. Experimental design was modulated in 25 rats divided into two groups: G1 that contained 10 rats as a control group and G2 contained 15 rats received 0.25% of the lethal dose (LD_{50}) of dioxins orally [9].

2-Dioxin Standard: Congeners labeled (17) with C^{13} and 17 native congeners at equal preparation was delivered from Chemishes und Verterinarunt-ersuchungsant reiburg, Germany. It is a stock standard solution containing pg WHO-TEO (TCDDs/PCDFs) of 17 congeners labeled with C^{13} and 17 native congeners at equal preparation. The initial concentration of Dioxin congeners were 1µL contain 10 pg of 17 active congeners.

3-Treatment of rats: Experimental animals received 50 µL from standard of dioxins. Dose calculated as a function of the body weight, hence 1 µL dioxin stock contains 10 pg of 17 congeners. These doses were applied to female rats using a stomach tube daily for one week. This dose was equal to 0.25 of LD_{50} of dioxin. After one week of treatment blood samples were collected and rats were sacrificed.

Blood samples were collected on heparin for investigating some biochemical and biophysical parameters. Samples were centrifuged at 800 rpm for 10 min at 4°C and plasma was removed. Packed cells were washed with 5 volume of saline at 20°C. This step was repeated three times. Packed cells were lysed with two volume of deionized water and the mixture was centrifuged at 5000 rpm for 30 min at 4°C to obtain the hemoglobin solution on which the experiments were carried out.

4-Determination of magnetic susceptibility: Magnetic susceptibility of hemoglobin is measured using the well known Alert method [23]. The force excreted on the sample tube in the magnetic field is determined with a commercial semimicro-balance. This rests upon a mechanical stage, adjustable in two directions, which permit adjustment of the sample between the shoes. Thermal disturbances of the magnetic field (3 to 10 KG) are provided by a water-cooled Wiess magnet. The air gap is 3.8 cm. Fluctuations in the laboratory supply direct current voltage frequently call for a special supply for the magnet. The alternating current supply is fed via a magnetic stabilizer (constant voltage transformer) to an adjustable transformer and rectifier in a two-way rectifier stage. The magnet current can be controlled by the regulating transformer.

- AC source
- Thermocouple
- Variable transformer with control
- Two-way rectifier
- Magnet
- Semi-micro balance
- Power supply for the heating jacket
- Magnetic constant voltage supply.

Calculation: Volume magnetic susceptibility is given from

$$K_S = \frac{\delta S}{S}\frac{W}{\delta W}\frac{\rho}{d}K_W + [1 - \frac{\delta S}{S}\frac{W}{\delta W}\frac{\rho}{d}K_a$$

Where

K_S: volume magnetic susceptibility
δS: weight change of the sample in and outside the field
δW: same parameter of the water
S: weight of sample
W: weight of water
ρ: density of sample
d: density of water

K_W is the volume magnetic susceptibility of water at the temperature of the measurements.

$$K_W = -0.72145 \times 10\text{-}6 - 0.000108\ (t\text{-}20) \times 10^{-6}$$

K_a is the volume magnetic susceptibility of air which is 0.029×10^{-6}

Molar magnetic susceptibility is given from:

Molar Mag. Sus. = $K_S \times$ molecular weight

5-Determination of heme concentration: Hemoglobin was converted to globin and heme using 20 ml of HCl-acetone (3:10 v/v) solution. Hemoglobin was introduced through the solution drop by drop, the mixture was vigorously stirred by a magnetic stirrer for 15 min. Heme was obtained by centrifugation for 10 min. at 2500 rpm. Heme was dissolved in 20 nM NaOH, stirred well in the dark for at least 10 min, diluted with an appropriate volume of water to achieve a desired concentration of 2 mM hemin in 5 mM NaOH and the solution centrifuged to separate undissolved materials. Hemin concentration was determined in 5 mM NaOH using an extinction coefficient 58.4 at 385 nm when application in Beer-Lamber law.

6-Determination of auto-oxidation rate: Measurement of auto-oxidation rate was carried out spectrophotometrically as described by [24] in air saturated 0.1 M phosphate buffer of pH 7.05 with 2 mg mL^{-1} HbO_2. The reaction was following the absorbance at fixed wavelength, usually at 630 nm, as a function of time hence, the rate by which the abnormal function of hemoglobin (Met-Hb) is a function of the absorbance peak at the characteristic wave length 630 nm as mentioned by Beer-Lamber law.

The pH was checked before and after each experiment and spectra of hemoglobin were recorded in order to confirm the absence of hemichrome during auto-oxidation rate measurements.

7-Determination of hemoglobin derivatives concentrations: The millimolar extinction coefficients were put into four linear equations with the four unknown concentrations of hemoglobin pigments (C-HbO_2, CHbCO, C-MetHb and C-SHb).

$$A^{500} = 5.05\ C_{HbO_2} + 5.35\ C_{HbCO} + 9.04\ C_{Met.Hb} + 7.2\ C_{SHb} \quad (1)$$

$$A^{569} = 11.27\ C_{HbO_2} + 14.27\ C_{HbCO} + 4.1\ C_{Met.Hb} + 8.1\ C_{SHb} \quad (2)$$

$$A^{577} = 15.37\ C_{HbO_2} + 10.0\ C_{HbCO} + 4.1\ C_{Met.Hb} + 8.1\ C_{SHb} \quad (3)$$

$$A^{620} = 0.24\ C_{HbO_2} + 0.33\ C_{HbCO} + 3.35\ C_{Met.Hb} + 20.8\ C_{SHb} \quad (4)$$

Where the absorption bands at wavelengths 500, 569, 577 and 620 nm represent the absorption maxima of Met-Hb, HbCO, HbO_2 and SHb, respectively [25]

This linear system of equations was solved by mathematical manipulation, using the Gaussian elimination method. For matrix calculation to yield the following equations:

$$C_{SHb} = \frac{A^{620} - 0.442293A^{500} + 0.1065519A^{569} + 0.0515769A^{577}}{18.895404} \quad (5)$$

$$C_{Met.Hb} = \frac{9.0602343A^{500} - A^{577} - 2.6960235A^{569} - 35.295898C_{SHb}}{66.750821} \quad (6)$$

$$C_{HbCO} = \frac{A^{569} - 2.2316831A^{500} + 16.074415C_{Met.Hb} + 7.9681188C_{SHb}}{2.330495} \quad (7)$$

$$C_{HbO_2} = \frac{A^{500} - 5.35C_{HbCO} - 9.04C_{Met.Hb} - 7.2C_{SHb}}{5.05} \quad (8)$$

Where A_{500}, A_{569}, A_{577} and A_{620} are the absorbances of hemoglobin solution at the wavelengths 500, 569, 577 and 620 nm, respectively.

8-Determination of Super Oxide Dismutase (SOD) activity: Determination of super oxide dismutase (SOD) activity was carried out by a RANDOX kit package [26].

9-Determination of Glutathione peroxidase activity: Determination of glutathione peroxidase activity is carried out by a RANDOX kit package [27].

Data were analyzed statistically by application of t-test to determine the significances of the results.

RESULTS

Table 1. showing the different hemoglobin concentrations in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin as. Significant($P<0.05$) increases in all abnormal hemoglobin derivatives were determined as compared to the control group. A significant($P<0.01$) decrease was recorded regarding the oxy-hemoglobin form.

Significant ($P<0.01$) decrease of the negative value of the molar hemoglobin magnetic susceptibility is shown in 2,3,7,8-tetrachlorodibenzo-p-dioxin treated rats compared to control (Table2).

Table 1: Hemoglobin of different ligand derivatives of rats received dioxin (Mean±SE)

Group	S-Hb	Met-Hb	Hb-CO	HbO_2
Control	0.534±0.03	1.41±0.52	2.41±0.086	92.15±0.27
Experimental	0.761±0.02*	2.01±0.47**	3.85±0.086**	86.78±0.23**

* Significant at P<0.05 ** Significant at P<0.01

Table 2: Hemoglobin molar magnetic susceptibility of rats received dioxin (Mean±SE)

Group	Magnetic susceptibility
Control	-0.974±0.025
Experimental	-0.694±0.067**

* Significant at P<0.05 ** Significant at P<0.01

Table 3: Molar heme concentration of rats received dioxin (Mean±SE)

Group	Molar heme concentration
Control	3.58±0.17
Experimental	5.47±0.24*

* Significant at P<0.05 ** Significant at P<0.01

Table 4: Glutathione peroxidase and Super Oxide Dismutase (SOD)

Group		Glutathione peroxidase (U/ml)	SOD activity (U/ml)
Control	Control	3787.61±864.41	112.5±9.48
Experimental	G-I	8218.33±927.53 **	154.07±5.27 **

* Significant at P<0.05 ** Significant at P<0.01

Measuring the molar heme concentration showed a significant ($P<0.05$) decrease after application of 2,3,7,8-tetrachlorodibenzo-p-dioxin as compared to control (Table 3).

Antioxidant enzymes activity obviously show a highly significant ($P<0.01$) increase either in superoxide dismutase or glutathione peroxidase in rats received 2,3,7,8-tetrachlorodibenzo-p-dioxin than those represented the control group (Table 4).

Fig. 1 obviously shown the difference between the hemoglobin absorption spectrum of rats received 2,3,7,8-tetrachlorodibenzo-p-dioxin as compared to the control.

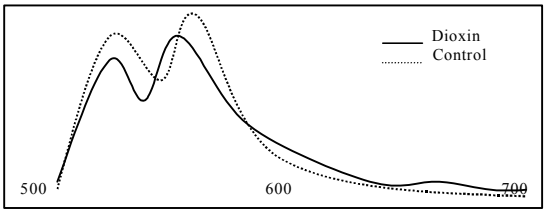

Fig. 1: Hemoglobin absorption spectrum of rats received dioxin

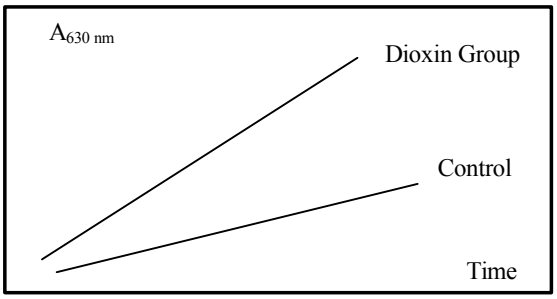

Fig. 2: Hemoglobin auto-oxidation rate of rats received dioxin

The most characteristic feature in the curve represent dioxin group is the appearance of a new peak at 630 nm that was characteristic to the formation of a new form of hemoglobin that is already oxidized by O_2.

Plotting of the relation between the absorbance value of the hemoglobin spectrum at wavelength 630 nm against time is shown as the auto-oxidation rate of hemoglobin (Fig. 2), the sharper the line,the higher the auto-oxidation rate is, that is very clear in the line represent the dioxin group.

DISCUSSION

Free radicals are defined as chemical species that possess one or more unpaired electrons. The term "Reactive Oxygen Species (ROS)" collectively described free radicals such as $O_2^{\bullet -}$, $OH^{\bullet}$ and other non-reactive oxygen derivatives such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) [28]. Formation of free radical in toxicity situations is confirmed by many reports. Oxidative stress is considered a possible molecular mechanism involved in toxicity [29, 30]. These ROS are generated by metabolic processes and their concentrations can be increased by environmental stimuli. To prevent ROS from damaging cellular components, organisms have evolved multiple detoxification mechanisms e.g. super oxide dismutase and glutathione peroxidase:

$$2\ O_2^{\bullet -} + 2\ H^+ \xrightarrow{\textit{Super}\ \text{Oxide Dismutase}} H_2O_2 + O_2$$

$$ROOH + 2\ GSH \xrightarrow{\textit{Glutathione}\ \text{peroxidase}} ROH + H_2O + GSSG$$

The obtained data in this study showed that the antioxidant activity increased concomitant with exposure to dioxin. This increased activity could be attributed to the higher formation rate of free radicals in this group, with higher consumption rate of antioxidants. Also, this increase in the antioxidant activity coincides with data obtained from measurement of the different hemoglobin derivatives concentrations. Whereas, an increase in all forms of abnormal hemoglobin derivatives (S-Hb, CO-Hb and Met-Hb) was evident. These derivatives increases as a result of the oxidation processes of the normal oxyhemoglobin in which the iron atom (ferrous state) is able to carry out its normal function as carrying oxygen and carbon dioxide to and from lungs and tissues. On the other hand, concentration of oxy-hemoglobin showed a dramatic decrease in animals received dioxin, a finding that may explained by lowering in the oxygen affinity of hemoglobin in cases of dioxin toxicity.

Elevated met-hemoglobin concentration is shown again in the absorption spectrum of hemoglobin (Fig. 1), a new peal at 630 nm which is specific to met-hemoglobin is obviously shown in spectrum of the animal received dioxin.

A normal physiological function of hemoglobin is the reversible binding of oxygen which can occur with the heme iron in the reduced (ferrous) state. In normal human, hemoglobin undergoes auto-oxidation to met-hemoglobin (oxidized form) at a rate of about 3% per day. Normally the methemoglobin can be restored to a functional form through reductive processes that take place in the red cell to maintain a maximum level of methemoglobin about 1% of the total hemoglobin. Met-hemoglobin reductase is the enzyme involved in this process [31]. The rate of normal dissociation of oxyhemoglobin to methemoglobin is highly dependent on the tertiary and quaternary structure of hemoglobin molecule. The higher rate of auto-oxidation (Fig. 2) in animals received TCDD may be related to the higher ROS formation rate.

Iron in hemoglobin derivatives is found in various electronic structures depending on both its oxidation state and on the group which occupies the sixth legend position. Magnetic susceptibility of hemoglobin is one of the most common properties that give a true picture of the molecule magnetic state. The sign and magnitude of the magnetic susceptibility can be used to give an approximate classification of the material and this can be summarized as follow:

K<0 Diamagnetic K>0 Paramagnetic and K>>0 Ferromagnetic

So, higher negative value of the molar magnetic susceptibility moving the hemoglobin molecule toward

the diamagnetic property in which the molecule has a higher molecular oxygen affinity. In this state iron atom can carry out the normal transition between higher Fe^{+++} to lower state Fe^{++} normally as a basic phenomena to change its oxygen and carbon monoxide affinity.

A non-significant increase in the molar heme concentration is recorded in rats received dioxin, this increase could be explained by one of the following ways (1) in toxic hemoglobin the tertiary structure of the molecule is unbalanced to the extent of affecting the linkage between globin moiety and heme atom so this force may be broken down releasing free molar heme (2) after the maturation of the globin moiety, its sterochemical structure is dramatically affected that in role affects the heme affinity of these globin moieties, so the binding between them is essentially absent [32].

Finally, it was concluded that 2,3,7,8-tetrachlorodibenzo-p-dioxin has a great effect on the magnetic properties of hemoglobin by shifting its magnetic susceptibility to the ferromagnetic region in which it cannot carry out its normal functions. 2,3,7,8-tetrachlorodibenzo-p-dioxin also plays a role in the hemoglobin oxidation process that may resulted in formation of non-functional hemoglobin forms. These findings are concomitant with increasing the auto-oxidation rate of hemoglobin to a level normal body antioxidants cannot control it.

REFERENCES

1. Poland, A. and J.C. Knutson, 1982. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanism of toxicity. Annual Review of Pharmacological Toxicology, 22: 517-554.
2. Landi, M.T., P.A. Bertazzi, A. Baccarelli, D. Consonni, S. Masten, G. Lucier, P. Mocarelli, L. Needham, N. Caporaso and J. Grassman, 2003. TCDD-mediated alterations in the AhR-dependent pathway in Seveso, Italy, 20 years after the accident. Carcinogenesis, 24: 673-680.
3. Ema, M, K. Sogawa, N. Watanabe, Y. Chujoh, N. Matshushita, O. Gotoh, Y. Funae and F. Kuriyama, 1992. cDNA cloning and structure of mouse putative Ah receptor. Biochemical Biophysical Research Communication, 184: 246-253.
4. Young, A.L., 1983. Human and environmental risks of chlorinated dioxins and related compounds. Plenumarly press: New York, pp: 173-190.
5. Lin, P., S.W. Hu and T.H. Chang, 2003. Correlation between gene expression of aryl hydrocarbon receptor (AhR), hydrocarbon receptor nuclear translocator (Arnt), cytochromes P4501A1 (CYP1A1) and 1B1 (CYP1B1) and inducibility of CYP1A1 and CYP1B1 in human lymphocytes. Toxicological Science, 71: 20-26.
6. Debdas Mukerjee, 1997. Health impact of polychlorinated dibenzo-p-dioxins: A critical review. Journal of the Air Management Association, 48: 157-165.
7. Crofts, F., E. Taioli, J. Trachman, G.N. Cosma, D. Currie, P. Toniolo and S.J. Garte, 1994. Functional significance of different human CYP1A1 genotypes. Carcinogenesis, 15: 2961-2963.
8. Theelen, R.M., 1991. Biological basis for risk assessment of dioxins and related compounds; Cold Spring harbor laboratory press; New York, pp: 277-290.
9. Poiger, H. and C. Schlatter, 1986. Health Impact of polychlorinated dibenzo-p-dioxins: A critical Review. Chemosphere, 1489-1494.
10. Nhats, F.Y., 1987. United States Environmenta; Protection Agency Office of Toxic Substances; Washington, DC; EPA 560/5-91-003.
11. Patterson, D.G., G.D. Todd, W.E. Turner, V. Maggio, L.R. Alexander and L.L. Needham, 1994. Environmental Health Perspect, 101: 195-204.
12. Alsharif, N.Z., W.J. Schlueter and S.J. Stohs, 1994b. Stimulation of NADPH-dependent reactive oxygen species formation and DNA damage by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat peritoneal lavage cells. Arch. Environmental Contamination Toxicology, 26: 392-397.
13. Muhammadpour, H., W.J. Murray and S.J. Stohs, 1988. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced lipid peroxidation in genetically responsive and non-responsive mice. Archeive of Environmental Contamination Toxicology, 16: 645-650.
14. Alsharif, N.Z., T. Lawson and S.J. Stohs, 1994. Oxidative stress induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin is mediated by the aryl hydrocarbon (Ah) receptor complex. Toxicology, 92: 39-51.
15. Shertzer, H.G., D.W. Nebert, A. Puga, M. Ary, D. Sonntag, K. Dixon, L.J. Robinson, E. Cianciolo and T.P. Dalton, 1998. Dioxin causes a sustained oxidative stress response in the mouse. Biochem. Biophysical Research Communication, 253: 44-48.

16. Hassoun, E.A., S.C. Wilt, M.J. DeVito, A. Van Birgelen, N.Z. Alsharif, L.S. Birnbaum and S.J. Stohs, 1998. Induction of oxidative stress in brain tissues of mice after subchronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicological Science, 42: 23-27.
17. Park, J.Y., M.K. Shigenaga and B.N. Ames, 1996. Induction of cytochrome P4501A1 by 2,3,7,8-tetrachlorodibenzo-p-dioxin or indolo(3,2-b)-carbazole is associated with oxidative DNA damage. Proceeding of National Academic Science, 93: 2322-2327.
18. Nebert, D.W., A. Puga and V. Vasiliou, 1993. Role of the Ah receptor and the dioxin-inducible [Ah] gene battery in toxicity, cancer and signal transduction. Annual New York Academic Science, 685: 624-640.
19. Tritscher, A.M., A.M. Seacat, J.D. Yager, J.D. Groopman, B.D. Miller, D. Bell, T.R. Sutter and G.W. Lucier, 1996. Increased oxidative DNA damage in livers of 2,3,7,8-tetrachlorodibenzo-p-dioxin treated intact but not ovariectomized rats. Cancer Letter, 98: 219-225.
20. Bondy, S.C. and S. Naderi, 1994. Contribution of hepatic cytochrome P450 systems to the generation of reactive oxygen species. Biochemical Pharmacology, 48: 155-159.
21. Kuthan, H. and V. Ullrich, 1982. Oxidase and oxygenase function of the microsomal cytochrome P450 monooxygenase system. European Journal of Biochemistry, 126: 583-588.
22. Morehouse, L.A. and S.D. Aust, 1988. Generation of superoxide by the microsomal mixed-function oxidase system. Basic Life Science, 49: 517-521.
23. Albert, Y. and J. Banerjee, 1975. Magnetic susceptibility measurements of deoxygenated hemoglobins and isolated chains. Biochemical and Biophysical Acta, 405: 144-154.
24. Guillochon, D., E. Laurent and T. Daniel, 1986. Effect of glutaraldehyde on hemoglobin oxidation-reduction potentials and stability. Biochemical Pharmacology, 35: 317-24m
25. Amira, A.S., M.R.. Bassem and M.M. Salem, 2006. New multicomponent spectrophotometric method for estimation of five hemoglobin derivatives in normal and traffic policemen. The Medical Journal of Cairo University, June, 74(2): 97-99.
26. McCord, J.M. and I. Fridovich, 1969. Method of SOD measurements Journal of Biological Chemistry, 244: 6049.
27. Wendel, A., 1980. Enzymatic basis of detoxification.Academic Press, N.Y., 333: 9013-66-5.
28. Vaziri, N.D., Y. Ding and Z. Ni, 2001. Compensatory up-regulation of nitric-oxide synthase isoforms in lead-induced hypertension; reversal by a superoxide dismutase-mimetic drug. Journal of Pharmacological Experimental Theory, 298: 679-685.
29. Machartova, V., J. Racek, J. Kohout, V. Holecek, I. Krejcova and V. Senft, 1998. Indicators of effects of free radicals in workers at risk of lead exposure. Vnitr Lek, 44: 83-85.
30. Moreira, E.G., G.J. Rosa, S.B. Barros, V.S. Vassilieff and I. Vassillieff, 2001. Antioxidant defense in rat brain regions after developmental lead exposure Toxicology, 14: 145-151.
31. Gary, A., R. Rockwood and I. Steven, 2003. Species Comparison of Methemoglobin Reductase. Baskin Experimental Biology and Medicine, 228: 79-83.
32. Sawka, M.N., V.A. Convertino, E.R. Eichner, S.M. Schnieder and A.J. Young, 2000. Blood volume: importance and adaptations to exercise training, environmental stresses and trauma/ sickness. Medical Scientific Sports Exerc., 32: 332-348.

(Received: 17/4/2008; Accepted: 24 /5 /2008)