BIOMEDICAL AND ENVIRONMENTAL SCIENCES 16, 95-104 (2003)

Ozone Emitted During Copying Process – A Potential Cause of Pathological Oxidative Stress and Potential Oxidative Damage in the Bodies of Operators

JUN-FU ZHOU*,[1], WEI-WEI CHEN*, AND GUI-ZHONG TONG#

*The Second Affiliated Hospital, College of Medicine, Zhejiang University, 88 Jiefang Road, Hangzhou 310009, Zhejiang, China; #Zhejiang Provincial Health and Epidemic Prevention Station, 17 Laozhedazhi Road, Hangzhou 310009, Zhejiang, China

Objective To estimate the impact of copying on the indoor air quality, and to investigate whether ozone emitted during such a process induces pathological oxidative stress and potential oxidative damage in the bodies of operators. **Methods** 67 copying operators (CO) and 67 healthy volunteers (HV) were enrolled in a random control study, in which levels of lipoperoxide (LPO) in plasma and erythrocytes, and levels of vitamin C (VC), vitamin E (VE) and β-carotene (β-CAR) in plasma as well as activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) in erythrocytes were determined by spectrophotometric methods. **Results** Compared with the HV group, the average values of LPO in plasma and erythrocytes in the CO group were significantly increased ($P<0.0001$), while those of VC, VE and β-CAR in plasma as well as those of SOD, CAT and GPX in erythrocytes in the CO group were significantly decreased ($P<0.0001$). Pearson product-moment correlation analysis showed that with increase of ozone level in copying sites and duration of exposure to ozone, the values of LPO in plasma and erythrocytes in the bodies of operators were gradually increased,while those of VC, VE, β-CAR, SOD, CAT and GPX were decreased in the same manner. Odds ratio (*OR*) of risk of biochemical parameters reflecting potential oxidative damage of the copying operators ranged from 4.440 to 13.516, and 95 % CI of *OR* was from 2.113 to 34.061. Reliability coefficient (α) of the biochemical parameters used to reflect the potential oxidative damage of the operators was 0.8156, standardized item $\alpha=0.9929$, $P<0.0001$. **Conclusion** Findings in the present study suggest that there exist a series of free radical chain reactions and pathological oxidative stress induced by high dose ozone in the operators, thereby causing potential oxidative and lipoperoxidative damages in their bodies.

Key Words: Ozone; Oxidation; Lipoperoxidation; Antioxidant; Antioxidase; Oxidative stress; Oxidative damage; Copying; Copying operators; Copier

INTRODUCTION

Copying of references, documents, information and photos plays important roles in social, economic, business, scientific and technological affairs, however, photochemical smog emitted during this process may become risk factors inducing potential oxidative

[1]Correspondence should be addressed to Prof. Jun-Fu ZHOU, Second Affiliated Hospital of College of Medicine, Zhejiang University, 88 Jiefang Road, Hangzhou 310009, Zhejiang, China. Tel: 86- 571-87783768, Fax: 86-571-87213864, E-mail: zhuojun88@sina.com

Biographical note of the first author: Jun-Fu ZHOU, M.D., research scientist and professor, male, born in 1945, graduated from Zhejiang Medical University, having more than 200 papers published and having won 22 awards of science and technology advances from the government and PLA. Research interests: preventive medicine and free radical medicine.

0895-3988/2003
CN 11-2816

damage in operators' bodies[1,2]. Some authors reported that laser printers and photocopiers emitted significant amounts of ozone, organic volatiles and formaldehyde during printing or operating process in a badly-ventilated office environment, thus leading to oxidative stress and potential oxidative damage of operators' bodies[1,2]. However, up to now, there are neither reports on changes of free radical reactions in the bodies of copying operators who are working in the badly-ventilated office environments, nor reports about relationship between copying and potential oxidative damage induced by ozone. To estimate the impact of copying process on the indoor air quality, and to investigate whether ozone emitted during this process induces pathological oxidative stress and potential oxidative damage in the bodies of operators, 67 copying operators (CO) and 67 healthy volunteers (HV) were enrolled in a random control study[3,4], in which levels of lipoperoxide (LPO) in plasma and erythrocytes, and levels of vitamin C (VC), vitamin E (VE) and β-carotene (β-CAR) in plasma as well as activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) in erythrocytes were determined by spectrophotometric methods. At the same time, differences between average values of biochemical parameters in the CO group and the HV group were compared, and Pearson product-moment correlation between ozone level in copying sites and the biochemical parameters as well as the correlation between duration of exposure to ozone and the biochemical parameters were analyzed. In addition, *odds ratio (OR) of risk of the biochemical parameters used to reflect the potential oxidative* damage of the copying operators, and 95% CI of *OR* were estimated, and reliability analysis of the biochemical parameters used to reflect the potential oxidative damage of the operators was processed.

MATERIALS AND METHODS

Subjects

Printing Sites. Thirty printing sites without well-ventilated equipment were used as samples. Their floor space ranged from 20 to 50 (31.6 ± 9.1) m^3, and there were 1-3 copiers in each site.

Copying Operators (CO). Sixty-seven copying operators (CO) were randomly sampled from 134 operators in some libraries and copying shops with "Select Cases-random Sample" of "SPSS 11.0 for Windows". Forty males and 27 females aged from 21 to 40 (28.3 ± 4.8) years. Their duration of exposure to ozone ranged from 2 to 10 (5.9 ± 2.5) years. They were all volunteers in this study.

Healthy Volunteers (HV). Sixty-seven healthy volunteers (HV) were randomly sampled from 200 healthy volunteers confirmed by comprehensive physical examination at the Second Affiliated Hospital, College of Medicine, Zhejiang University with "Select Cases-random Sample" of "SPSS 11.0 for Windows". Thirty three males and 24 females aged from 21 to 40 (28.9 ± 4.9) years. They had no link with copy-printing operation, and were all volunteers in this study.

There was no significant difference between the average values for ages by t test (t= 0.1530, P=0.8787) as well as between the gender proportions in the two groups by x^2 test (x^2 =0.5658, P=0.4519).

Medical history of disorders associated with brain, heart, lung, liver, kidney and other organs as well as blood, circulatory, respiratory, digestive and other systems of the operators and the healthy volunteers were all excluded by their routine blood, urine and feces examinations as well as radiographs, cardiogram and other necessary tests. And their

medical history of any episode of inflammation, hypertension, hyperlipidemia, acute or chronic bronchitis, autoimmune disease, diabetes, atherosclerosis, tumors and other diseases, and subnutrition, malnutrition, supernutrition and other nutritional diseases were also excluded. In addition, there were no smoking and excessive drinking history in the above subjects.

The copying operators and healthy volunteers had never been exposed to radiation, or engaged in work with exposure to intoxicating materials or pesticides. Within the preceding month when they were enrolled as volunteers in the study, none of them had taken any antioxidant supplements such as vitamin C, vitamin E, β-carotene, ginkgo biloba, tea polyphenols or other similar substances.

Methods

Measurement of Ozone Level in the Indoor Air of Copying Sites

An ozone-analyzer was used to determine directly ozone level in the indoor air in 30 copying sites. The level of ozone was measured on 150 cm above the ground at four corners in each site for 1.0 min at 10 a.m. and 3 p.m., and its average value was expressed as mg/m^3.

Collection and Pretreatment of Blood Samples

Fasting venous blood samples were collected in the morning and heparin sodium was added as anticoagulant, and the plasma and erythrocytes promptly separated were stored at −50℃ immediately[3]. The blood samples did not undergo any hemolysis.

Measurement of Every Biochemical Constituent

The methods used in determining various biochemical constituents were outlined below, with details provided in references[3]. The spectrophotometry of thiobarbituric acid reactive substances (TBARS) was used to determine plasma LPO level expressed as μmol/L[3]. The spectrophotometry of TBARS was used to determine erythrocytic LPO level expressed as nmol/g.Hb[3]. The spectrophotometry of ferrozine coloration was used to determine plasma VC and VE levels expressed all as μmol/L[3]. The plasma β-CAR was extracted with a mixture of ethanol and petroleum ether, and assayed with spectrophotometry, with its level expressed as μmol/L[3]. The spectrophotometry of inhibiting pyrogallol auto-oxidation was used to determine erythrocytic SOD activity expressed as U/g.Hb[3]. The spectrophotometry of coloration of hydrogen peroxide and acetic acid-potassium dichromate was used to determine erythrocytic CAT activity expressed as K/g.Hb[3]. The improved Hafeman's spectrophotometry was used to determine erythrocytic GPX activity expressed as U/mg.Hb[3].

In the determination of the above biochemical substances and enzymes, the main analytical reagents, such as vitamin C, vitamin E, β-carotene, 5,6-diphenyl-3-(2-pyridyl)-1, 2,4-triazinedisulfonic acid disodium salt (ferrozine), Cu/Zn-superoxide dismutase, catalase, 1,2,3-trihydroxybenzene (pyrogallol), 1,1,3,3-tetraethoxypropane, 2-thiobarbituric acid, were all purchased from SIGMA® Chemical Company, USA; and the other analytical reagents were all produced in China. The fresh quadruply distilled water was prepared with a quartz glass distilling apparatus. In the determination of the above biochemical substances and enzymes, the main analytical instruments included HP 8453-Spectrophotometer, USA, and UV-754-Spectrophotometer and 721-Spectrophotometer. The instrument used to determine the ozone level was LIDA DCS-1 Ozone-analyzer, Shanghai LIDA Instrument Factory, China.

In the determination of the above biochemical substances and enzymes, all experiments were standardized by using the same batch number of each reagent, the same quality control and the same lab assistant, and the identical analytical apparatus and instruments were

strictly applied for every experiment in order to control and decrease its errors and bias, and to ensure its analytical quality[3,4].

Medical Statistical Analysis

All data were statistically analyzed with SPSS 11.0 for Windows statistic software using a Compaq Pentium Ⅳ/1.6 GHz computer. The biochemical parameters in this study presented all normal distributions by Kolmogorov-Smirnov test and Shapiro-Wilk test, and they were expressed as mean plus or minus standard deviation ($\bar{x}\pm s$) and 95% confidence interval (95% CI). Hypothesis testing methods included independent-samples *t* test, Pearson chi-square test (x^2 test), Pearson product-moment correlation analysis, estimate of odds ratio (*OR*) of risk and 95 % CI of *OR*, and reliability analysis. In the statistical analysis of this study, the level of hypothesis testing (α) was $\leqslant 0.05$ in order to avoid false positives (α-error), and the power of hypothesis testing (*power*) was $\geqslant 0.80$ to avoid false negatives (β-error)[3,4].

RESULTS

Level of Ozone in the Indoor Air in the Printing Sites

The level of ozone in the indoor air in 30 printing sites without well-ventilated equipment ranged from 0.106 to 0.401 (0.224 ± 0.082) mg/m^3. Pearson product-moment correlation analysis showed that the level of ozone in the indoor air was increased with the decrease of floor space of the site, $r = -0.5534$, $t = 3.5162$, $P = 0.000273$).

Comparison Between the Average Values ($\bar{x}\pm s$) of the Biochemical Parameters in the CO Group and the HV Group

Compared with the average values of the biochemical parameters in the HV group, the average values of LPO in plasma and erythrocytes in the CO group were significantly increased ($P<0.0001$), while those of VC, VE and β-CAR in plasma as well as those of SOD, CAT and GPX in erythrocytes in the CO group were significantly decreased ($P<0.0001$) (Table 1).

95% CI of the Average Values of the Biochemical Parameters in the CO Group and the HV Group

The lower limits of 95% CI of the average values of LPO in plasma and erythrocytes in the CO group were greater than the upper limits of 95% CI of those in the HV group. The upper limits of 95% CI of the average values of VC, VE and β-CAR in plasma as well as SOD, CAT and GPX in erythrocytes in the CO group were less than the lower limits of 95% CI of those in the HV group (Table 1).

Pearson Product-moment Correlation Analysis Between the Ozone Level in the Indoor Air of the Copying Sites and Each Biochemical Parameter in 67 Copying Operators

The findings of Pearson product-moment correlation analysis showed that the levels of LPO in plasma and erythrocytes in the copying operators were increased with the elevation of ozone level in the indoor air of the sites, while those of VC, VE and β-CAR in plasma as well as those of SOD, CAT and GPX in erythrocytes in the operators were decreased with the elevation of ozone level in the indoor air there (Table 2).

TABLE 1

Comparison Between the Average Values ($\bar{x}\pm s$) of the Biochemical Parameters in the CO Group and in the HV Group

Group	n	Oxidative Constituents		Antioxidative Constituents					
		Plasma	Erythrocyte	Plasma			Erythrocyte		
		LPO (μmol/L)	LPO (nmol/g.Hb)	VC (μmol/L)	VE (μmol/L)	β-CAR (μmol/L)	SOD (U/g.Hb)	CAT (K/g.Hb)	GPX (U/mg.Hb)
CO	67	12.41±1.82 (11.97-12.86)	32.65±4.77 (31.49-33.81)	38.32±9.73 (35.95-40.69)	16.47±4.17 (15.45-17.48)	1.24±0.33 (1.16-1.32)	1875.8±147.6 (1839.8-1911.8)	258.5±67.4 (242.0-274.9)	21.36±5.96 (19.91-22.81)
HV	67	10.87±1.62 (10.47-11.27)	28.37±4.10 (27.37-29.37)	54.26±14.09 (50.83-57.70)	24.49±6.19 (22.98-26.00)	1.72±0.46 (1.61-1.83)	2047.8±162.5 (2008.2-2087.4)	317.6±83.3 (297.3-338.0)	28.73±8.02 (26.78-30.69)
t^*		5.1628	5.5690	7.6234	8.8012	6.8869	6.4122	4.5195	6.0390
P		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Note: *Independent-samples t test. The figures in parentheses are confidence limits.

TABLE 2

Pearson Product-moment Correlation Analysis Between the Ozone Level in the Indoor Air of the Copying Sites and the Biochemical Parameters

Correlative Item	*n*	*r*	*t*	*P*
Ozone Level With Plasma LPO	67	0.4648	4.2318	0.000074
Ozone Level With erythrocytic LPO	67	0.4142	3.6689	0.000493
Ozone Level With Plasma VC	67	−0.5261	4.9874	0.000005
Ozone Level With Plasma VE	67	−0.4180	3.7093	0.000433
Ozone Level With Plasma β-CAR	67	−0.5021	4.6814	0.000015
Ozone Level With Erythrocytic SOD	67	−0.4460	4.0175	0.000155
Ozone Level With Erythrocytic CAT	67	−0.4526	4.0920	0.000120
Ozone Level With Erythrocytic GPX	67	−0.4522	4.0878	0.000122

Pearson Product-moment Correlation Analysis Between the Duration of Exposure to Ozone and Each Biochemical Parameter in 67 Copying Operators

The findings of Pearson product-moment correlation analysis showed that the levels of LPO in plasma and erythrocytes in the copying operators were increased with the duration of exposure to ozone, while those of VC, VE and β-CAR in plasma as well as those of SOD, CAT and GPX in erythrocytes in the copying operators were decreased with the duration of exposure to ozone (Table 3).

TABLE 3

Pearson Product-moment Correlation Analysis Between the Duration of Exposure to Ozone and the Biochemical Parameters

Correlative Item	*n*	*r*	*t*	*P*
Duration of Exposure to Ozone With Plasma LPO	67	0.4167	3.6955	0.000452
Duration of Exposure to Ozone With Erythrocytic LPO	67	0.4870	4.4959	0.000029
Duration of Exposure to Ozone With Plasma VC	67	−0.3334	2.8511	0.005833
Duration of Exposure to Ozone With Plasma VE	67	−0.3390	2.9051	0.005013
Duration of Exposure to Ozone With Plasma β-CAR	67	−0.3432	2.9455	0.004470
Duration of Exposure to Ozone With Erythrocytic SOD	67	−0.4472	4.0312	0.000148
Duration of Exposure to Ozone With Erythrocytic CAT	67	−0.4193	3.7236	0.000413
Duration of Exposure to Ozone With Erythrocytic GPX	67	−0.4205	3.7371	0.000395

Estimate of the Odds Ratio (OR) of Risk of the Biochemical Parameters Used to Reflect the Potential Oxidative Damage in the Copying Operators, and 95 % CI of OR

Supposing the values of LPO in plasma and erythrocytes in the CO group $\geqslant$ the upper limits of 95% CI of those in the HV group to be the reference criteria of positive number of potential oxidative damage of the copying operators, and supposing the values of VC, VE and β-CAR in plasma as well as SOD, CAT and GPX in erythrocytes in the CO group $\leqslant$ the lower limits of 95 % CI of those in the HV group to be the reference criteria of positive number of potential oxidative damage of the copying operators, the odds ratio (*OR*) of risk of the biochemical parameters used to reflect the potential oxidative damage in the copying operators ranged from 4.440 to 13.516, and 95% CI of *OR* ranged from 2.113 to 34.061 (Table 4).

TABLE 4

Estimate of the Odds Ratio of Risk (*OR*) of the Biomedical Parameters Reflecting Potential Oxidative Damages in Copying Operators, and 95 % CI of *OR*

Group	*n*	Plasma		Erythrocyte		Plasma						Erythrocyte					
		LPO		LPO		VC		VE		β-CAR		SOD		CAT		GPX	
		+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
CO	67	50	17	50	17	58	9	60	7	56	11	55	12	51	16	54	13
HV	67	20	47	19	48	28	39	26	41	28	39	24	43	28	39	28	39
Odds Ratio of Risk (95% CI)		6.912 (3.235-14.770)		7.430 (3.458-15.966)		8.976 (3.822-21.078)		13.516 (5.364-34.061)		7.091 (3.159-15.914)		8.212 (3.691-18.268)		4.440 (2.113-9.327)		5.786 (2.663-12.572)	
x^2		26.920		28.712		29.215		37.525		25.013		29.637		16.314		21.244	
P		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	

Note. "+" indicates "number cases" that the values of LPO in plasma and erythrocytes in the CO group $\geqslant$ the upper limits of 95 % CI of those in the HV group, and that the values of VC, VE, β-CAR, SOD, CAT and GPX in the CO group $\leqslant$ the lower limits of 95% CI of those in the HV group, while "−" means the opposite.

Reliability Analysis for the Biochemical Parameters Used to Reflect the Potential Oxidative Damage in the Copying Operators

The findings of reliability analysis of the ozone level in the indoor air in the copying sites without well-ventilated equipment and the levels of LPO in plasma and erythrocytes, and the levels of VC, VE and β-CAR in plasma as well as the activities of SOD, CAT and GPX in erythrocytes used to reflect the potential oxidative damage in the copying operators were as follows: the reliability coefficient (α) = 0.8156, and the standardized item α = 0.9929, $P < 0.0001$.

DISCUSSION

LPO and its metabolic products, such as malondialdehyde and conjugated diene, etc., play an important role in the metabolism of the human body[3,5-18]. VC, VE, β-CAR, SOD, CAT and GPX are the most important antioxidants in the human body[3,5-18]. Significant increase of LPO level and marked decrease of antioxidant levels in the human body may cause metabolic disorders and pathological aggravation of a series of free radical chain reactions, thus inducing a variety of diseases related to abnormal reactions of free radicals [3,5-18].

The findings in the present study show that there exists serious ozone pollution in the indoor air in the copying sites without well-ventilated equipment, even though the lowest ozone level is beyond the recommended maximum exposure limit of 0.1 mg/m^3 in the hygienic standard of public places, and far exceeds the ozone olfact (olfactory threshold of ozone) of 0.018 mg/m^3 [20]. The findings also show that there exists an imbalance between oxidation and antioxidation, and a pathological oxidative stress and potential free radical damage in the copying operators.

The simplest reason is that a large amount of ozone emitted during copying process could not be adequately exhausted to the open air because of badly-ventilated office environments. Ozone is one of the main components and the most abundant oxidant in the photochemical smog[1,2,20-23]. Ozone may cause oxidative decomposition and peroxidative modification of polyunsaturated fatty acids, unsaturated phospholipids, glycolipids and cholesterol in plasma and cell membranes, which may induce aggravation of oxidation, peroxidation and lipoperoxidation in the human body, thus resulting in marked increase of LPO in the copying operators who have inhaled a large amount of ozone, and leading to potential free radical damage in the operators' bodies[20,22,24]. In addition, significant decrease and/or loss of GPX activity induced by DNA damage derived from high ozone level may also result in acute lipoperoxidation[3,7-9,15,16,19,20,22,24], and such lipoperoxidation may lead to lipoperoxidative damage of cells, and to cytoclasis[20,22,24]. At the same time, the high dose ozone and ozonizing process in the human body may generate a large amount of superoxide anion radicals (O_2^-) hydxoyl radicals (·OH), hydroperoxyl radicals ($HO_2^·$) and other free radicals[26], which may also cause oxidative decomposition and peroxidative modification of many organic compounds in the human body, thereby further producing a large amount of LPO[3,7-9,15,16,19,20,22,24].

As a strong oxidant, ozone or its reactive products may interact directly with DNA, thus causing DNA damage, inhibiting or depressing DNA replication[20,22]. Ozone may also linearize circular DNA, and induce ozone-sensitive mutant[20,22], which leads to a significant decrease in synthesis or regeneration of SOD, CAT and GPX, and results in marked decrease

of their activities. In addition, excessive O_2^-, ·OH and other free radicals as well as single oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and other reactive oxygen species may also cause DNA damage and attack strongly the structures of VC, VE, β-CAR, SOD, CAT, GPX and other antioxidants by combining them with some active groups, thereby inactivating these antioxidants[3,7-9,15,16,19,20,22,24,26].

The findings of Pearson product-moment correlation analysis in the present study show that the levels of the above biochemical constituents in the copying operators are closely related to the ozone level in the indoor air in the copying sites, and to the duration of exposure to ozone[27]. Ozone level in the indoor air should, therefore, be controlled and kept below the recommended maximum exposure limit by a well-ventilated office environment in any copying site[1,2,19].

The odds ratio of the risk estimate in the present study suggests that compared with the healthy volunteers, the risk inducing potential oxidative damage in the copying operators will increase by a factor of 3.4-12.5, and that photochemical smog, especially ozone, has very strong oxidative power, thus causing pathological oxidative stress and potential oxidative damage in the human body[1,2,19-30]. At the same time, the reliability analysis in the study further shows that the high dose ozone plays a very important role in pathological oxidative stress and potential oxidative damage in the copying operators.

In summary, the findings in the present study suggest that there exist a series of free radical chain reactions and pathological oxidative stress induced by high dose ozone in the copying operators, thereby causing potential oxidative damage and lipoperoxidative damage in their bodies. We, therefore, recommend that there should be well-ventilated equipment in any copying site so that ozone concentration in the indoor air may be decreased below 0.1 mg/m^3, and that antioxidant vitamins at suitable doses, such as vitamin C, vitamin E and β-carotene, should be given to copying operators daily in order to alleviate potential oxidative damage and lipoperoxidative damage in their bodies because these antioxidant vitamins are good free-radical scavengers and have a protective effect against photo-oxidant exposure damage of ozone[3,7-9,15,16,19,28-30].

REFERENCES

1. Tuomi, T., Engstrom, B., Niemela, R., Svinhufvud, J., and Reijula, K. (2000). Emission of ozone and organic volatiles from a selection of laser printers and photocopiers. *Appl. Occup. Environ. Hyg.* **15**, 629-634.
2. Brown, S. K. (1999). Assessment of pollutant emissions from dry-process photocopiers. *Indoor. Air.* **9**, 259-267.
3. Zhou, J. F., Yan, X. F., Guo, F. Z., Sun, N. Y., Qian, Z. J., and Ding, D. Y. (2000). Effects of cigarette smoking and smoking cessation on plasma constituents and enzyme activities related to oxidative stress. *Biomed. Environ. Sci.* **13**, 44-55.
4. Greenberg, R. S., Daniels, S. R., Flanders, W. D., Eley, J. W., and Boring, J. R. (2001). *Medical Epidemiology (3rd ed.)*, New York, McGraw-Hill.
5. Murray, R. K. (2000). Muscle and the cytoskeleton. In *Harper's Biochemistry* (Eds. Murray, R. K., Granner, D. K., Mayes, P. A., and Rodwell, V. W., 25th ed.), pp. 715-736. New York, McGraw-Hill Press.
6. Zhou, J. F., Yan, X. F., Ruan, Z. R., Peng, F. Y., Cai, D., Yuan, H., Sun, L., Ding, D. Y., and Xu, S. S. (2000). Heroin abuse and nitric oxide, oxidation, peroxidation, lipoperoxidation. *Biomed. Environ. Sci.* **13**, 131-139.
7. Zhou, J. F., Chen, P., Yang, J. L., Zhu, Y. G, Peng, C. H., and Wu, Y. L. (2000). Oxidative stress before and after operation in patients with chronic cholecystitis containing gallstone. *Biomed. Environ. Sci.* **13**, 254-262.
8. Zhou, J. F., Chen, J. X., Shen, H. C., and Cai, D. (2002). Abnormal reactions of free radicals and oxidative damages in the body of patients with chronic glomerulonephritis. *Biomed. Environ. Sci.* **15**, 233-244.
9. Mayes, P. A. (2000). Biologic oxidation. In *Harper's Biochemistry* (Eds. Murray, R. K., Granner, D. K., Mayes, P. A., and Rodwell, V. W., 25th ed.), pp. 130-136. New York, McGraw-Hill Press.
10. Mayes, P.A. (2000). Lipidds of physiologic significance. In *Harper's Biochemistry* (Eds. Murray, R. K., Granner, D. K., Mayes, P. A., and Rodwell, V. W., 25th ed.), pp. 160-171. New York, McGraw-Hill Press.
11. McKee, T., and McKee, J. R. (2000). *Biochemistry: An Introduction* (2nd ed.), pp. 289-330. New York,

McGraw-Hill Press.
12. Lingappa, V.R., and Farey, K. (2000). *Physiological Medicine: A Clinical Approach to Basic Medica Physiology* (1st ed.), pp. 25-30. New York, McGraw-Hill Press.
13. Thuraisingham, R. C. and Raine, A. E. G. (1998). Endocrine manifestations of systemic disease: the heart. In *Clinical endocrinology* (Ed. Grossman, A., 2nd ed.), pp. 1077-1082. Victoria, Blackwell Science Ltd Press.
14. Zhou, J. F. and Chen, P. (2001). Studies on the oxidative stress in alcohol abusers in China. *Biomed. Environ. Sci.* **14**, 180-188.
15. Chen, H.H. and Zhou, J.F. (2001). Low cholesterol in erythrocyte membranes and high lipoperoxides in erythrocytes are the potential risk factors for cerebral hemorrhagic stroke in human. *Biomed. Environ. Sci.* **14**, 189-198.
16. Chen, P. and Zhou, J.F. (2001). Changes of oxidative and lipoperoxidative metabolism in patients with acute viral myocarditis. *Journal of Zhejiang University (SCIENCE)* **2**, 448-452.
17. Xie, B., Zhou, J. F., Lu, Q., Li, C. J., and Chen, P. (2002). Oxidative stress in patients with acute coxsackie virus myocarditis. *Biomed. Environ. Sci.* **15**, 48-57.
18. Zhou, J. F., Xu, G. B., and Fang, W. J. (2002). Relationship between acute organophosphorus pesticide poisoning and damages induced by free radicals. *Biomed. Environ. Sci.* **15**, 177-186.
19. Dong, S.H. (1995). The public sanitation and its management. In *Modern Environmental Hygiene* (Ed. Cai, H. D., 1st ed.), pp. 1005-1006. Beijing, People's Medical Publishing House. (In Chinese)
20. Pavlovic, M., Alebic-Juretic, A., Klasinc, L., Trinajstic, N., Turk, R., and Kezele, N. (1994). Man, the environment and ozone. *Arh. Hig. Rada. Toksikol.* **45**, 275-284.
21. Kelly, F. J., Mudway, I., Krishna, M.T., and Holgate, S.T. (1995). The free radical basis of air pollution: focus on ozone. *Respir. Med.* **89**, 647-656.
22. Mudway, I.S., Housley, D., Eccles, R., Richards, R.J., Datta, A.K., Tetley, T.D., and Kelly, F. J. (1996). Differential depletion of human respiratory tract antioxidants in response to ozone challenge. *Free. Radic. Res.* **25**, 499-513.
23. Steinberg, J. J., Gleeson, J. L., and Gil, D. (1990). The pathobiology of ozone-induced damage. *Arch, Environ. Health.* **45**, 80-87
24. Girotti, A. W. (1998). Lipid hydroperoxide generation, turnover, and effector action in biological systems. *J. Lipid. Res.* **39**, 1529-1542.
25. Hamada, A. (1995). Chlorination and ozonation of contaminants in water environment and role of free radicals. *Yakugaku. Zasshi.* **115**, 790-804
26. Mickalad, A.M., Hans, P., Dupont, G.D., Hoebeke, M., Deby, C., and Lamy, M. (1998). Propofol reacts with peroxynitrite to form a phenoxyl radical: demonstration by electron spin resonance. *Biochem. Biophys. Res. Commun.* **249**, 833-837.
27. Avissar, N.E., Reed, C.K., Cox, C., Frampton, M.W., and Finkelstein, J.N. (2000). Ozone, but not nitrogen dioxide, exposure decreases glutathione peroxidases in epithelial lining fluid of human lung. *Am. J. Respir. Crit. Care. Med.* **162** (4 Pt 1), 1342-1347.
28. Romieu, I., Meneses, F., Ramirez, M., Ruiz, S., Padilla, R. P., Sienra, J. J., Gerber, M., Grievink, L., Dekker, R., Walda, I., and Brunekreef, B. (1998). Antioxidant supplementation and respiratory functions among workers exposed to high levels of ozone. *Am. J. Respir. Crit. Care. Med.* **158**, 226-232.
29. Grievink, L., Zijlstra, A.G., Ke, X., and Brunekreef, B. (1999). Double-blind intervention trial on modulation of ozone effects on pulmonary function by antioxidant supplements. *Am. J. Epidemiol.* **149**, 306-314.
30. Weber, S.U., Han, N., and Packer, L. (2001). Ozone: An emerging oxidative stressor to skin. *Curr. Probl. Dermatol.* **29**, 52-61.

(*Received January* 28, 2002 *Accepted January* 7, 2003)