Skin diseases and enzymatic antioxidant activity among workers exposed to pesticides

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الأمراض الجلدية والفعالية الإنزيمية المضادة للأكسدة في العاملين المعرضين لمبيدات الهوامّ

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الخلاصة: تمت دراسة 150 عاملاً ممن يتعرضون لمبيدات الهوامّ (الآفات) و50 فرداً من الأصحّاء باعتبارهم شواهد، وذلك بالفحص السريري (الإكلينيكي) والجلدي واختبار اللصاقات الجلدية واختبارات وظائف الكلية والكبد، وتعداد خلايا الدم، وسكر الدم وتحليل البول. كما تم تقييم فعالية الإنزيمات المضادة للأكسدة التالية: ديسموتاز البيروكسيد وبيروكسيداز الغلوتاثيون ومختزلة الغلوتاثيون. وقد كانت الموجودات الجلدية إيجابية لدى 78٪ من المتعرضين للفوسفات العضوية و76٪ من المتعرضين لمركبات البيريثرويدات و54٪ لمركبات الكاربامات. وقد كان اختبار اللصاقة إيجابياً لدى 70٪ من العاملين الذين تعرضو لمركبات البيريثرويدات و64٪ من المتعرضين لمبيدات الهوام من الكاربامات. ويزداد مستوى الإنزيمات الكبدية بشكل عام لدى العاملين مع نقص فعالية الإنزيمات المضادة للأكسدة نقصاً شديداً في جميع العاملين بالمقارنة مع الشواهد.

ABSTRACT In this study, 150 workers exposed to pesticides and 50 healthy control subjects were given clinical and dermatological examinations, patch tests, tests of liver and renal function, complete blood count, blood sugar and urinalysis. Activity of the antioxidant enzymes superoxide dismutase, glutathione peroxidase and glutathione reductase was also evaluated. Dermatological findings were positive in 78%, 76% and 54% of workers exposed to organophosphates, pyrethroids and carbamate pesticides respectively. The patch test was positive in 70% of workers exposed to pyrethroids and 64% exposed to carbamate pesticides. Liver enzyme levels were generally increased in workers while antioxidant enzyme activity was significantly decreased in all workers compared with the controls.

Les dermatoses et l'activité enzymatique antioxydante chez les travailleurs exposés aux pesticides
RESUME Dans cette étude, on a réalisé des examens cliniques et dermatologiques, des tests épicutanés, des tests de la fonction hépatique et rénale, un hémogramme, une épreuve de glycémie et des analyses d'urine chez 150 travailleurs exposés à des pesticides et 50 sujets témoins en bonne santé. L'activité des enzymes antioxydants superoxyde dismutase, glutathion-peroxydase et glutathion-réductase a également été évaluée. Les résultats des examens dermatologiques étaient positifs chez 78 %, 76 % et 54 % des travailleurs exposés aux pesticides organophosphorés, aux pyréthroïdes et aux carbamates respectivement. Le test épicutané était positif chez 70 % des travailleurs exposés aux pyréthroïdes et chez 64 % de ceux qui étaient exposés aux carbamates. Il y avait généralement une élévation des niveaux d'enzyme hépatique chez les travailleurs tandis que l'activité enzymatique antioxydante était considérablement réduite chez tous les travailleurs par rapport aux sujets témoins.

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Introduction

A pesticide is a generic term that covers a wide range of compounds employed in pest control, including insecticides, fungicides, herbicides, algicides, acaricides and avicides. These compounds may contain chlorinated hydrocarbons, organophosphorus compounds, organomercury compounds, pyrithrine and gamma benzene hexachloride. Approximately 90% of current pesticides are organic compounds. Insecticides consist primarily of organochlorine compounds, organophosphate compounds, and carbamate compounds [*1*].

Employees in the pesticide industry and pest control frequently experience dermal exposure to pesticides, with subsequent absorption through the skin. The rate at which a pesticide is absorbed through the skin is determined by the nature of the compound, the condition of the skin, and external factors such as temperature. The most rapid and complete absorption occurs with pesticides that have some solubility in both water and lipids [*2*].

A variety of toxicological effects have been reported in workers dealing with pesticides. Chloracne, dermatitis on prolonged skin contact, porphyria, liver disorders, and neurological and behavioural changes are the more frequently reported clinical findings [*3,4*].

Pesticides may increase oxidative damage. They may be metabolized to free radicals, e.g. paraquat is reduced to a free radical that reoxidizes to make a superoxide and regenerate paraquat, which accumulates selectively in the lung. The postulated defensive role of antioxidants against the activities of free radicals in the body has become an area of much recent interest. Superoxide dismutases are found in mitochondria and cytosol and convert superoxides to hydrogen peroxide. If there is an increase in the production of free radicals in the body, extra antioxidants are also produced. However, if large numbers of extra free radicals are produced, cell damage and death may result from the imbalance between free radicals and antioxidants [*5*].

Pesticides are still widely used in agricultural and non-agricultural settings in Egypt. Data on the prevalence of skin diseases, liver disorders, renal disorders and the activity of antioxidant enzymes following pesticide exposure are limited. The incidence of skin diseases related to pesticides is predicted to be high, since the amount of pesticide used per unit area in Egypt is large. Furthermore most pesticide users are unaware of the adverse effects of pesticides on human health and thus do not follow the instructions on their proper use.

In the present study, the adverse effects of pesticides on the skin and other body systems were studied in workers handling pesticides. The activities of the antioxidant enzymes, superoxide dismutase, glutathione peroxidase and glutathione reductase, were also evaluated.

Methods

The study was conducted on 150 workers who had been exposed to pesticides. These workers regularly prepared and/or applied pesticides. All were men, with a mean age of 23 ± 2 years. As controls, 50 healthy non-exposed men were also examined. All the workers and controls came from six cities in Sharkia Governorate, Egypt.

All workers were given a questionnaire including a personal history, full occupational history and the total duration of exposure to pesticides, type of pesticide used, and relation of exposure to the pesticide and any skin disease if present. All workers

and controls underwent clinical and dermatological examinations. Laboratory investigations, including complete blood picture, fasting and postprandial blood glucose, liver function tests, renal function tests and urinalysis, were completed for every worker at the start of the study and after a follow-up period of 2 years.

According to the type of pesticide exposure, workers were divided into 3 groups:

- Group I: exposed to diazinone (organophosphate group)
- Group II: exposed to rup (pyrethroid group)
- Group III: exposed to Sevin (carbamate group).

Patch test

All workers were patch tested for the three pesticides used. The patch test preparations were obtained as pure chemicals from the Ministry of Agriculture and prepared by the researchers. The test agents were applied to Finn chambers (Epitest, Helsinki, Finland), which were fixed to the upper part of the back with Scanpor® Tape and secured with 3M™ tape. The patches were removed after 48 hours and the sites were examined for evidence of reaction. The sites were examined again at 72 hours. The reading at 72 hours was considered to be positive if the reaction was equal to or stronger than a palpable erythema.

Liver function tests

Serum glutamate oxalacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase, serum bilirubin and serum proteins were assayed according to Tietz [7].

Kidney function tests

Blood urea and serum creatinine were assayed according to Tietz [7].

Activity of antioxidant enzymes [8]

Antioxidant enzymes were assayed spectrophotometrically using an enzymatic method (Randox Laboratories Limited., County Antrim, United Kingdom).

Superoxide dismutase [8]

The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical (O_2^-), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. This assay method uses xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye, read spectrophotometrically at 340 nm. SOD activity is then measured by the degree of inhibition of this reaction.

Heparinized whole blood samples were collected. Whole blood (0.5 mL) was centrifuged for 10 minutes at 3000 rpm and the supernatant plasma was collected. Erythrocytes were washed four times with 3 mL of 0.9 sodium chloride solution, centrifuged for 10 minutes at 3000 rpm after each wash. The washed centrifuged erythrocytes were made up to 2.0 mL with cold redistilled water, mixed and left to stand at 4 °C for 15 minutes. The lysate was diluted with 0.1 mmol/L phosphate buffer pH 7.0 so that the percentage inhibition fell between 30% and 60%.

Glutathione peroxidase and reductase [8]

Glutathione peroxidase (GPX) catalyses the oxidation of glutathione (GSH) by cumene hydroperoxidase. In the presence of glutathione reductase (GR) and NADPH, oxidized glutathione (GSSG) is immediately converted to the reduced form, with a concomitant oxidation of NADPH to NADP+.

The decrease in absorbance at a wavelength of 340 nm is measured.

Glutathione reductase catalyses the reduction of glutathione in the presence of NADPH, which is oxidized to NADP+. The decrease in absorbance at a wavelength of 340 nm is measured.

Heparinized whole blood samples were centrifuged for 5 minutes at 2000 rpm. The plasma and buffy coat were removed. The erythrocytes were washed three times by resuspending in 0.9% sodium chloride solution, centrifuging for 5 minutes at 2000 rpm after each wash. The cells were lysed by resuspending in cold redistilled water and left for 10 minutes at 20 °C to 8 °C. The lysate was then centrifuged for 5 minutes at 2000 rpm to remove stroma. 100 μl of lysate was diluted with 1.9 mL of 0.9% sodium chloride solution for assay.

Statistical analysis

The results were expressed in terms of the mean and standard deviation. Different groups were compared by ANOVA. The level of significance was $P < 0.05$.

Results

Our study included 150 workers exposed to pesticides and 50 healthy normal controls. All workers were regularly handling pesticides. Of these, 120 (80%) were regularly spraying pesticides while 30 (20%) prepared the pesticides. Table 1 shows the ages of the workers and controls, while Table 2 shows the duration of exposure to the three pesticides examined in this study. Table 3 shows the dermatological diseases observed in the workers and controls. Contact dermatitis was found in 8 (16%) in group I, 12 (24%) in group II and 6 (12%) in group III. Dermatitis was seen on the volar aspects of fingers and on the palms, and was more prevalent on the right hand. Dryness of skin was reported in 18%, 14% and 14% of the workers in groups I, II and III respectively. Dryness of skin was found mainly on the face, and on the dorsum of the hands, feet and legs. Dermographism (in which stroking the skin with a pointed object produces erythema, pruritus and a linear wheal) was observed in 20% of workers exposed to Rup (group II), 16% of workers in group I and 18% of workers in group III. Hair loss represented a problem in all three groups (6% in each). Pigmentation of the skin, especially on the areas exposed to the sun, was reported in 8%, 4% and 4% of group I, group II and group III respectively. Chloracne consists of straw-coloured cysts, comedones, pustules and abscesses, and was reported by 12% in group I and 8% in group II, but not reported in group III. Porphyria was reported only by one worker in group I. Few of these conditions were found in the controls and the differences was statistically significant in the case of contact dermatitis, dermographism and chloracne ($P < 0.05$).

Table 4 shows the effect of pesticides on other body systems. Cough was reported by 20% in group I, 14% in group II and 4% in group III. Bronchial asthma on expo-

Table 1 **Age of exposed workers and controls**

Age group (years)	Group I ($n = 50$)	Group II ($n = 50$)	Group III ($n = 50$)	Controls ($n = 50$)
20–29	5	8	4	10
30–39	15	13	17	15
40–49	18	14	12	12
50+	12	15	17	13

Table 2 **Duration of exposure to pesticides in worker groups**

Duration (years)	Group I ($n = 50$) No.	%	Group II ($n = 50$) No.	%	Group III ($n = 50$) No.	%
5–14	19	38.0	12	24.0	11	22.0
15–24	20	40.0	25	50.0	23	26.0
25+	11	22.0	13	26.0	16	32.0
Total	50	100.0	50	100.0	50	100.0

Table 3 **Dermatological findings in the worker and control groups**

Dermatological findings	Group I ($n = 50$) No.	%	Group II ($n = 50$) No.	%	Group III ($n = 50$) No.	%	Controls ($n = 50$) No.	%	χ^2	*P*-value
Contact dermatitis	8	16	12	24	6	12	0	0	13.26	0.0041*
Dryness of skin	9	18	7	14	7	14	3	6	3.36	0.3393
Dermographism	8	16	10	20	9	18	1	2	8.31	0.0401*
Hair loss	3	6	3	6	3	6	0	0	3.14	0.3703
Pigmentation	4	8	2	4	2	4	1	2	2.21	0.5298
Chloracne	6	12	4	8	0	0	0	0	11.37	0.0098*
Porphyria	1	2	0	0	0	0	0	0		

**Significant at P < 0.05.*

Table 4 **Other clinical findings in the worker and control groups**

Other clinical findings	Group I ($n = 50$) No.	%	Group II ($n = 50$) No.	%	Group III ($n = 50$) No.	%	Controls ($n = 50$) No.	%	χ^2	*P*-value
Cough	10	20	7	14	2	4	3	6	8.38	0.0388
Asthma	4	8	3	6	2	4	0	0	4.07	0.2537
Impaired vision	0	0	4	8	4	8	0	0	8.83	0.0396*
Arthralgia	5	10	0	0	0	0	3	6	9.38	0.0246*
Myalgia	6	12	4	8	6	12	4	8	0.89	0.8281

**Significant at P < 0.05.*

sure to the pesticides was reported by 8%, 6% and 4% in group I, II and III respectively. Impaired vision and red eyes were found in 8% of workers in group II and group III. Arthralgia was only reported by 10% of workers in group I, while myalgia was found in 12%, 8% and 12% of workers in group I, II and III respectively. The results showed a statistically significant difference compared to the controls in the case of impaired vision and arthralgia ($P < 0.05$).

All the workers and controls were patch-tested with a series of locally used pesticides (Table 5). Significantly more workers had a positive patch test than the controls. Pyrethroids were the most frequent sensitizer, followed by carbamates and organophosphates.

The results of biochemical laboratory tests in the groups are presented in Table 6. Serum proteins, serum bilirubin and liver enzymes were significantly increased in pesticide-exposed workers in all three groups compared to the controls ($P < 0.05$). Alkaline phosphatase, serum creatinine, blood urea, blood sugar and urinalysis results were all within normal values.

The activities of antioxidant enzymes are presented in Table 7. The mean values ± standard deviation of SOD were 157.97 ± 25.04 U/mL, 153.96 ± 23.05 U/mL and

Table 5 **Patch test in the worker and control groups**

Patch test	Group I ($n = 50$) No.	%	Group II ($n = 50$) No.	%	Group III ($n = 50$) No.	%	Controls ($n = 50$) No.	%	χ^2	*P*-value
Positive	17*	34	35*	70	32*	64	0	0	63.55	0.000*
Negative	33	66	15	30	18	36	50	100		

**Significant at P < 0.05*

Table 6 **Biochemical laboratory tests in the worker and control groups**

Tests	Group I ($n = 50$) Mean ± *s*	Group II ($n = 50$) Mean ± *s*	Group III ($n = 50$) Mean ± *s*	Controls ($n = 50$) Mean ± *s*
Serum proteins (g/dL)	71.18 ± 0.7*	8.12 ± 0.5*	6.19 ± 0.4*	6.88 ± 0.69
Serum bilirubin (mg/dL)	0.73 ± 0.19*	0.80 ± 0.12*	0.63 ± 0.11*	0.50 ± 0.13
SGOT (U/L)	16.30 ± 6.56*	18.30 ± 6.6*	15.20 ± 7.0*	7.93 ± 2.0
SGPT (U/L)	13.50 ± 5.3*	14.50 ± 5.6*	17.50 ± 4.4*	7.01 ± 1.3
Alkaline phosphatase	5.90 ± 1.32	6.20 ± 1.35	5.80 ± 1.32	5.90 ± 2.19
Serum creatinine (mg/dL)	0.56 ± 0.32	0.42 ± 0.32	0.6 ± 0.31	0.56 ± 0.18
Blood urea (mg/dL)	27.40 ± 5.26	29.40 ± 6.26	28.40 ± 6.22	26.32 ± 4.2

**ANOVA test significant at P < 0.05.*
SGOT = serum glutamate oxalacetate transaminase.
SGPT = serum glutamate pyruvate transaminase.
S = standard deviation.

Table 7 **Activity of antioxidant enzymes in the worker and control groups**

Enzyme	Group I ($n = 50$) Mean ± s	Group II ($n = 50$) Mean ± s	Group III ($n = 50$) Mean ± s	Controls ($n = 50$) Mean ± s
SOD (U/mL)	157.97 ± 25.04*	153.96 ± 23.05*	155.66 ± 24.3*	200.71 ± 20.4
GPX (U/L)	1425.9 ± 419.15*	1415.2 ± 418.15*	1420.9 ± 414.5*	2082.07 ± 418.64
GR (U/L)	25.12 ± 8.26*	23.11 ± 7.29*	24.15 ± 7.75*	29.92 ± 17.88

**ANOVA test significant at P < 0.05.*
SOD = superoxide dismutase.
GPX = glutathione peroxidase.
GR = glutathione reductase.
s = standad deviation.

155.66 ± 24.3 U/mL in groups I, II, III respectively. GPX mean values were 1425.9 ± 419.15 U/L, 1415.2 ± 418.15 U/L, and 1420.9 ± 414. 5 U/L in group I, II and III respectively. GR mean values were 25.12 ± 8.26 U/L, 23.11 ± 7.29 U/L and 24.15 ± 7.75 U/L in group I, II and III respectively. There was a significant decrease in the activity of SOD, GPX and GR compared to controls (P <0.05).

Discussion

With many pesticides, the possibility of dermal exposure with subsequent absorption through the skin may present a greater problem to workers than does exposure through inhalation. However, both routes of absorption may occur concomitantly [*9*]. Pesticides produce several skin diseases in humans in three distinct stages: occupational exposure to the intermediate chemicals from manufacture in the factory, contact by spray operators using end products in agricultural fields, and contact by consumers with the contaminated products [*10*].

In this study many dermatological diseases related to exposure to pesticides were reported among the workers, with statistically significant differences compared to the healthy controls. Contact dermatitis was observed in workers exposed to organophosphates (diazinon), pyrethroids (Rup) and carbamate (Sevin), with a particularly high incidence in workers dealing with pyrethroids (24%). Contact dermatitis could be partially explained by sensitivity to the skin allergens in the pesticides as most workers did not wear adequate protective gloves and clothing. Close contact and skin exposure to pesticides are to be expected in these working conditions. Contact dermatitis has been reported by many other authors [*11–14*]. Stoianov et al. [*15*] explained dermatitis caused by organophosphorus pesticides in terms of their destructive effect on the ultrastructure of skin cells, on body metabolism and on enzyme systems. They also induce changes in polyunsaturated fatty acids. Another explanation for dermatitis was given by Sharkey et al., who suggest that pesticides stimulate the generation of superoxides by monocytes and of oxygen-derived free radicals by phagocytes at the site of tissue inflammation [*16*]. These cause initial immunological damage to the skin, fol-

lowed by dermatitis. In this study most of the workers developed dermatitis from prolonged and repeated exposure to relatively small quantities of pesticides. This produced diffuse, dry and lichenified eruptions without vesiculation.

Exposure to a low concentration of the pesticide can produce gradual damage to the horny layer, denaturing the keratin and affecting the water-holding capacity. Dryness of the skin was reported by 18%, 14%, and 14% in workers exposed to organophosphates, pyrethroids and carbamates respectively. Cumulative dermatitis is often preceded by a long period of dryness or chapping.

In this study dermographism was very prevalent among workers exposed to pyrethroids (20%), followed by carbamates (18%) and organophosphates (16%). In dermographism, stroking the skin with a pointed object produces erythema, pruritus and a linear wheal. Dermographism is produced by capillary dilatation, which leads to extravasation of proteins and fluid. Release of acetylcholine from nerve endings in the skin can cause vasodilatation and increase capillary permeability. Mast cells are very sensitive and are easily stimulated by toxic substances like pesticides to release inflammatory mediators, of which histamine, histamine-like substances and serotonin are the most important in inducing capillary dilatation [*17*].

Hair loss was equally prevalent in each group. Pesticides are rapidly taken up by anagen follicles. The result is disturbances in keratinization: many hairs break within the follicles, while other follicles enter catagen prematurely. Loss of hair is the most consistent symptom.

Skin pigmentation was particularly common in workers dealing with organophosphates. It may be due to exposure to sunlight, heavy manual work, or frequent minor trauma combined with exposure to the pesticide.

Chloracne was reported by 12% of workers exposed to organophosphates and 8% of workers exposed to pyrethroids. Toxicity may have occurred through direct contact, inhalation or ingestion. Similar findings were described by Jirasek [*18*].

In this study one worker exposed to organophosphates had symptom of porphyria cutanea tarda (PCT). PCT is characterized by a reduction in hepatic urodecarboxylase activity. Sporadic PCT may be precipitated by environmental factors, alcohol, oestrogen, iron or xenobiotics. Symptoms include weakness, photosensitivity, bullae, hypertrichosis and pigmentation. This result is in line with the results of Jirasek [*18*] and Altomare and Capella [*19*].

In this study the highest prevalence of skin disease was observed among workers exposed to organophosphates (78%), followed by those exposed to pyrethroids (76%) and finally those exposed to carbamates (54%). These results are consistent with those of Cellini and Offidani [*20*] and Guo et al. [*21*].

Cough and asthma were among the effects on other systems reported in this study. Hayes [*22*] speculated that about 25% of inhaled pesticide would be re-exhaled, about 50% would be deposited in the upper respiratory passages and subsequently swallowed, and about 25% would be deposited in the lower respiratory passages. This represents a significant occupational hazard.

Impaired vision was found in workers exposed to pyrethroids and carbamates. Ocular exposure is usually a result of accidental spills or splashes, but visual impairment following chronic exposure may also be important. A similar finding was reported by Ishikawa [*23*].

Arthralgia and myalgia result from inactivation or inhibition of the enzyme cholinesterase leading to accumulation of acetylcholine at synapses in the peripheral nervous system. Excess acetylcholine at the neuromuscular junction of skeletal muscles is the cause of weakness [*24*].

On patch testing, pyrethroids were the most frequent sensitizer, followed by carbamates and organophosphates. Similar results were obtained by Sharma and Kaur [*25*]. Pesticides should thus be patch-tested on all workers with contact dermatitis.

Finally, biochemical laboratory tests showed significant increases in liver function tests. Test results did not return to normal values within the follow-up periods. These findings are in agreement with those of Jirasek [*18*]. The mechanism underlying the hepatic toxicity of pesticides is unclear. Pesticides may indirectly block the synthesis of proteins. This might in turn lead to damage of organelles and plasma membranes of hepatic parenchymal cells, interfering with their function and allowing leakage of enzymes [*18*].

It is known that pesticides may irritate lung macrophages, encouraging them to generate the superoxide radical (O_2^-). Antioxidant enzymes are used by the organisms as natural endogenous protection against the generation of reactive oxygen species [*26*]. Superoxide dismutases are metalloenzyme scavengers, which destroy superoxide radicals by converting them into hydrogen peroxide and oxygen by dismutation [*27*]. However, the reaction product hydrogen peroxide must be removed, not only because it is mildly toxic and inactivates SOD enzymes, but also because it can form other more reactive and damaging free radical species, including the hydroxyl radical. SOD works in conjunction with two enzymes, glutathione peroxidase and catalase, that degrade hydrogen peroxide to water and oxygen [*28*]. Glutathione reductase converts oxidised glutathione back to glutathione [*26*]. The normal pro-oxidant/antioxidant balance of the cell can be shifted in favour of pro-oxidants when the production of oxygen species is increased (as following exposure to chemicals or drugs), or when levels of antioxidants are diminished. This state is called oxidative stress and can result in serious cell damage if the stress is massive or prolonged [*28*]. The decreased activity of antioxidant enzymes observed in this study can therefore be explained in several ways. Pesticides may be metabolized to an oxygen free radical that re-oxidizes to make superoxide; the pesticide may itself be a free radical; or the pesticide may deplete antioxidant defences. The overall effect of pesticide is the production of more free radicals. The activity of superoxide dismutase, glutathione peroxidase and glutathione reductase were decreased, due to consumption of these enzymes to neutralize free radicals generated by pesticides.

Conclusion

Contact dermatitis, dermographism, pigmentation, chloracne and porphyria were prevalent among workers. Impaired enzymatic antioxidant activity and liver function were also present. To minimize these effects, impermeable protective clothing must be used whenever skin contact is likely. Periodic measurements of antioxidant enzymes, biochemical laboratory tests, and patch tests should be made available to workers at shorter intervals. In addition, dietary antioxidants may help to reduce the lifetime cumulative effect of oxidative damage.

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