Polish Journal of Occupational Medicine and Environmental Health, Vol. 6, No 2, 157-167, 1993

METABOLIC DISPOSAL OF ANTIPYRINE AND LIPID PEROXIDATION INDICES IN VOLUNTEERS JOINTLY EXPOSED TO INDUSTRIAL ORGANIC SOLVENTS AND ETHANOL

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Key words: Toluene, Xylene, Ethanol, Combined exposure, Volunteers, Antipyrine test, Lipid peroxidation

Abstract: Each of twelve volunteers, at 2 week intervals, received 1 g of antipyrine, a test drug, and were exposed for 4 h either to toluene (375 mg/m^3) or xylene (435 mg/m^3) singly or in combination with ethanol (0.45 g/kg body wt. before the onset of exposure and 0.15 g/kg thrice every 1 h during exposure to maintain a steady level of ethanol in blood $\approx$ 11 mmol/dm^3). No significant differences were found in salivary antipyrine half-life ($T\frac{1}{2} \approx 12$ h); and clearance ($Cl_A \approx 0.83$ cm^3/s) between control and groups exposed to solvents and/or ethanol. Nevertheless, a tendency to increase the metabolic rate of antipyrine in xylene-exposed group ($T\frac{1}{2} \approx 6.8$ h; $Cl_{AP} \approx 1.40$ cm^3/s) and counteraction of ethanol ($T\frac{1}{2} \approx 15$ h; $Cl_{AP} \approx 0.63$ cm^3/s) should be noted. The stimulation of lipid peroxidation in the serum as a biological effect of combined exposure to ethanol and toluene/xylene was observed.

INTRODUCTION

Many workers who are occupationally exposed to organic solvents may also be consumers of alcoholic beverages occasionally during workday and particularly after workhours. Since the excretion of organic solvents is delayed by their high solubility and storage in lipid-rich tissue, it is not uncommon to find ethanol and solvents in the body simultaneously.

The general influence of ethanol on the metabolism of foreign compounds is well known from animal experiments and human medical data (14, 20). Ethanol in a single, high dose is known to inhibit the metabolism of foreign compounds and stimulate the process through induction of cytochrome P−450 monooxygenases when administered repeatedly (14, 24). On the other hand, animal experiments have

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indicated that many organic solvents can induce cytochrome P−450 monooxygenase and thereby increase the metabolic capacity of the liver. Among these are toluene (18, 31) and xylene (10, 30, 35). The inductive effects of xylene may be fortified by prolonged ethanol ingestion (35). However, in animal studies on the interaction between solvents and ethanol, the doses/concentrations of the solvents highly exceeded those of the interactions with occupationally used chemicals. In the occupational medicine and toxicology practice, the molar concentration of ethanol in blood after social drinking is at least 100−200 times higher than molar blood solvent concentration during relevant experimental or occupational exposure (an average ≈ 6 mmol/l of ethanol/l and 0.05 mmol of solvent/l for rats or 17 mmol/l and 0.02−0.05 mmol/l for human volunteers, respectively) (21, 22) and the difference of concentration is still much larger after termination of solvent exposure. Therefore, it can be expected that ethanol may primarily affect solvent metabolism rather than vice versa.

The ingestion by human volunteers of a moderate dose of ethanol (0.8 g/kg body wt. yielding blood level 20 mmol/l that is exceeding the limit for car driving) prior to inhalatory exposure to m-xylene at TLV level (100−400 mg/m3) raised the level of xylene in blood by about 2 fold and decreased by 50% excretion with urine m-hippuric acid, the predominant final metabolite (21). Kinetics of toluene in human volunteers was similarly affected by a single dose of ethanol (32, 33). Regular drinking of ethanol accelerated metabolic rate of toluene (32). The kinetic data, in comparable conditions, were confirmed in rats (22). These results suggest that single, moderate doses of ethanol slow down the metabolism of the aromatic solvent whereas continued ethanol intake increases the metabolism, presumably by inducing the microsomal cytochrome P-450 monooxygenase in the liver.

In order to prove this assumption by a non-invasive quantitative assessment adopted for humans, an indirect *in vivo* antipyrine test may be applied (6). This test has the advantage of being relatively resistant to influence by moderate alcohol consumption (below 30 g/day) (7) while changes in the antipyrine metabolic rate (half-life and clearance) have been found in workers exposed occupationally to some organic chemicals. For example, an increase in antipyrine clearance was found to be due to occupational exposure to toluene and phenol (17), and petroleum mixture (12). In workers exposed to carbon disulphide known for its inhibitory effect on microsomal monooxygenases (36) a decrease in antipyrine clearance was observed (16). The metabolic effect of occupational exposure to trichloroethylene as evaluated by the antipyrine test largely depends on the grade and purity of the degreasing solvent (27). Assessment of the antipyrine metabolic rate in workers occupationally exposed to toluene (8), styrene (5) and vinyl chloride (16) has not revealed any changes in salivary clearance and half-life of the test-drug. The well established difference in antipyrine clearance between smokers and non-smokers (about 20%) was, however, reproduced (9) as well as between healthy and hepatocirrhosis-affected subjects (16).

The adverse metabolic interactions by work-related chemicals is of concern because occupational exposure is generally far greater than any other environmental risk factor. However, there are exceptions to this, such as ethanol consumption, which is a well recognized extra factor that may contribute to increased health hazard from occupational exposure (20). It has been accepted that results from well designed studies with human volunteers can help to assess the human health hazard

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from industrial exposure that has been superimposed on concomittantly ingested ethanol.

The aim of this study was to assess the metabolic effects, with antipyrine as a probe, of a single, controlled inhalatory exposure of human volunteers to vapours of xylene and toluene at a concentration near the TLV (200–750 mg/m^3 for a majority of countries; (100 mg/m^3 for Poland) (1, 34) singly and in combination with ethanol ingestion at a moderate dose, yielding its blood level $\approx$ 11 mmol/l (former denomination 0.5%) taken as a threshold of intoxication in traffic regulations. The biological effect of single and combined exposure of volunteers to solvents and ethanol was evaluated on the basis of determination of lipid peroxidation products in the serum as a specific xenobiotics induced toxic effect through free radical formation.

MATERIALS AND METHODS

Subjects

The subjects of the study were twelve healthy male volunteers aged 22–44 years, weight 67–110 kg and height 165–186 cm. The study was conducted according to the ethical principles adopted by the World Medical Association (4) and the protocol was approved by the Ethical Committee of the University Medical Academy in Lodz. Each volunteer undervent a medical examination without pathological findings. An informed consent was obtained from every subject. Each one was medically examined prior to and after the exposures.

Exposure

The volunteers were divided into two groups: 7 (4 nonsmokers and 3 smokers) and 5 (3 nonsmokers and 2 smokers) persons each and at 2-week intervals they were exposed either to toluene (375 mg/m^3) or xylene (435 mg/m^3) in a dynamic chamber for 4 hours (9.00 a.m. – 1:00 p.m.) (laboratory grade POCh, Poland). The exposures were single or combined with ethanol (Rectified Spirit, POLMOS, Poland) ingestion. Ethanol was diluted to 20% (w/v) with an orange juice and given to drink at doses of 0.45 g/kg body wt. before and 0.15 g/kg thrice every 1 h during exposure to maintain a steady level of ethanol in blood $\approx$ 11 mmol/l. Before exposure, after a standarized breakfast, each of the volunteers received a 1 g of antipyrine (POLFA, Poland) in drinking water, a test drug for the evaluation of the metabolic capacity of the liver for xenobiotics *in vivo*. The corresponding reference controls received antipyrine and then were sham-exposed to air flow and drank ethanol or orange juice. A different exposure regimen took place at two-week intervals.

Collection of the saliva and blood samples

The saliva samples (1.5–2 ml) for antipyrine determination were taken prior to the exposure (0 h) and then at 4, 8 and 24 h from the onset of the exposure. The saliva samples were kept at 4°C until completion, then centrifuged and the super-

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natant was stored at −20°C and analysed within a month. The capillary blood samples for determination of ethanol were taken at 0, 2 and 4 h from the onset of exposure. The venous blood samples (≈ 4 ml) for determination of triglycerides and lipid peroxidation products were taken after the completion of exposure in the chamber.

Analytical methods

Concentration of toluene and xylene in the exposure chamber was calculated three times during the exposure period by means of gas chromatograph (Varian 1440). Ethanol in blood was determined enzymatically with alcohol dehydrogenase and NAD (2). Antipyrine in saliva was determined spectrophotometrically according to Brodie et al. (3) as modified by Dossing et al. (8) and the kinetics parameters of salivary antipyrine was calculated as follows:

(1) After determining the antipyrine concentrations in saliva, a curve of antipyrine loss over time was determined and then antipyrine elimination coefficients k (per h) were assigned according to the formula

$$k = \frac{\log C_{t_o} - \log C_{t_1}}{0.4343\ (t_1 - t_o)}$$

where: t_o and t_1 are selected times on the loss curve, while C_{t_o} and C_{t_1} are antipyrine concentrations in the saliva during t_o and t_1, respectively.

(2) The biological half-life of antipyrine $T\frac{1}{2}$ (in h) was calculated from the formula

$$T\tfrac{1}{2} = \frac{\ln 2}{k} = \frac{0.693}{k}$$

(3) For calculating the volume of antipyrine distribution V_D (in dm^3) an empirical formula for males was applied, where:

$$V_D = 0.3625 \times \text{body wt (kg)} + 0.2239 \times \text{body height (cm)} - 0.1387 \times \text{age (years)} - 14.47$$

(4) Antipyrine clearance Cl_{AP} (in cm^3 per sec) was calculated from the formula:

$$Cl_{AP} = \frac{k \times V_D \times 1000}{3600}$$

The level of triglycerides in the serum was determined spectrophotometrically with a standard kit of Boehringer-Mannheim Test-Combination.

Lipid peroxidation in the serum was evaluated on the basis of fluorimetric detection (excitation − 515 nm; emission − 553 nm) of thiobarbituric acid-reactive substances according to Yagi (37), comparing the fluorescence intensity of the sample with that of a standard solution of tetramethoxypropane (syn. malondialdehyde tetramethylacetal). The lipid peroxide level can be expressed in terms of malondialdehyde.

Statistical evaluation of the results was performed according to the Student's t-test.

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RESULTS

The kinetic parameters of salivary antipyrine in volunteers exposed to ethanol and/or industrial organic solvents, toluene and xylene, are presented in Tables 1–3.

Table 1. Evaluation of the activity of hepatic manooxygenase system on the basis of salivary antipyrine test in volunteers exposed to ethanol and/or toluene

Groups	Number of subjects	Ethanol in blood (mmol/dm^3)	Antipyrine in saliva Clearance (cm^3/s)	Antipyrine in saliva Half-life (h)
Control	7	1.30 ± 0.21	0.73 ± 0.06	13.3 ± 1.3
Ethanol	7	12.20 ± 1.52	0.73 ± 0.06	13.4 ± 1.6
Toluene	7	1.65 ± 0.21	0.68 ± 0.03	13.8 ± 0.9
Toluene + Ethanol	7	11.72 ± 0.87	0.70 ± 0.06	13.5 ± 0.6

Regimen of exposure: Ethanol, 20% water solution, given to drink at doses 0.45 g/kg body wt before and 0.15 g/kg twice every 1 h during 4-hour exposure to toluene (or exposure to air as reference); Toluene, inhalatory 4-hour exposure at concentration 375 mg/m^3 in a dynamic chamber with simultaneous intake of ethanol (or water as a reference); Antipyrine, a test drug, in a dose of 1 g was given before onset of the exposure. The corresponding reference controls, all dosed with antipyrine, were sham exposed to air flow and drunk ethanol or water. Salivary antipyrine concentration was determined at 0, 4, 8 and 24 h from the intake.
The levels of ethanol in blood were controlled at 2 and 4 h from the intake and the mean values presented. Results are the mean $\pm$SE.

Table 2. Evaluation of the activity of hepatic monooxygenase system on the basis of salivary antipyrine test in volunteers exposed to ethanol and/or xylene

Groups	Number of subjects	Ethanol in blood (mmol/dm^3)	Antipyrine in saliva Clearance (cm^3/s)	Antipyrine in saliva Half-life (h)
Control	5	1.95 ± 0.43	0.90 ± 0.10	11.2 ± 2.5
Ethanol	5	11.29 ± 1.09	0.93 ± 0.03	12.7 ± 1.9
Xylene	5	1.30 ± 0.21	1.40 ± 0.16**	6.8 ± 1.1**
Xylene + Ethanol	4	10.64 ± 0.86	0.63 ± 0.06	15.1 ± 2.6
Xylene + Ethanol *	1	37.8	0.20	42.3

Concentration of xylene in a dynamic chamber amounted 435 mg/m^3. Other details on regimen of exposure as in Table 1.
* Insubordinate volunteer drank alcohol on the day before; take notice that the metabolic rate of antipyrine dramatically decreased.
** Differences not proven ($0.05 < p < 0.10$) on account of too few volunteers tested. Results are the mean $\pm$SE.

As can be seen from the data of Table 1, ethanol at the level of 12 mmol/dm^3 of blood as well as toluene at a concentration of 375 mg/m^3 did not change the antipyrine clearance ($Cl_{AP} \approx 0.7$ cm^3/s) and half-life ($T^{1/2} \approx 13$ h); the combined exposure to both of the chemicals still did not affect the antipyrine kinetics. The effect of ethanol (blood level $\approx$ 11mmol/dm^3) and xylene (435 mg/m^3 for 4 h) on saliva antipyrine kinetics is presented in Table 2. As inferred from the data, ethanol at the delimiting level has no effect on the values of antipyrine clearance (0.90 cm^3/s) and half-life ($\approx$12 h) whereas xylene seems to exert a stimulating influence on the kinetics of salivary antipyrine, although only an insignificant tendency ($0.05 < p < 0.10$) was noted, probably on account of the small number of examined volunteers. This conclusion is supported by the fact that the combined exposure to ethanol and xylene abolished the quasi-stimulatory effects. Moreover, it happened that an insubordinate volunteer, drunk alcohol on the day before the experiment and

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Table 3. Decline of the concentration of antipyrine in saliva in the post-exposure period in volunteers exposed to ethanol and/or toluene/xylene

Groups	Number of subjects	Concentration of antipyrine in saliva in different times after dosing, μg/cm³			Volume of distribution V_D dm³
		4 h	8 h	24 h	
Control	12	13.73 ± 1.04	10.58 ± 0.80	4.29 ± 0.50	47.76 ± 2.21
Ethanol	12	13.32 ± 1.21	10.34 ± 1.17	4.50 ± 0.65	48.18 ± 2.36
Toluen	7	14.02 ± 0.52	10.58 ± 0.67	5.53 ± 0.48	49.50 ± 3.14
Toluen + Ethanol	7	14.79 ± 61.09	12.79 ± 0.81	5.85 ± 0.73	48.76 ± 2.81
Xylene	5	9.09 ± 1.47[a]	7.64 ± 0.80[a]	1.37 ± 0.47[c]	46.34 ± 3.81
Xylene	4	12.79 ± 2.83	10.03 ± 1.97	4.62 ± 1.12	47.03 ± 4.84
+ Ethanol	1*	22.48	15.43	16.19	43.58

* Insubordinate volunteer, with the level of ethanol in blood amounted to 37.8 mmol/dm³, threefold exceeding the delimiting level 12 mmol/dm³.

Control and ethanol groups were composed of the volunteers from concurrent reference groups of toluene- and xylene-exposure. Other details as in Tables 1 and 2.

a, c – significantly different from the control at p = 0.05 and 0.001, respectively.

during combined exposure to xylene + ethanol his extremely high level of ethanol in blood (≈ 38 mmol/dm³) resulted in complete inhibition of antipyrine elimination/metabolism yielding a clearance ≈ 0.20 cm³/s and half-life ≈ 42 h.

To prove the tendency of xylene in accelerating the metabolic rate of antipyrine, we performed an analysis of the concentration of the testing-drug in the saliva over the course of the post-exposure period at 4, 8 and 24 h for solvent-exposed groups of volunteers. The results were compared with the group of sham-exposed control and ethanol-exposed subjects (Table 3). It can be seen from Table 3 that time-course of elimination of antipyrine from saliva is markedly accelerated with time in the xylene-exposed subjects in comparison to the control and the differences in concentration at all tested time-points were statistically significant. Exposure to xylene superimposed on ethanol intake led to abolishing the stimulatory effect of xylene alone; an overdose of ethanol, by the insubordinate volunteer, almost completely inhibited the course of elimination of antipyrine.

Looking for the biological markers of the effect of single and combined exposure to ethanol and/or toluene/xylene, the level of lipid peroxidation products in the serum was determined at the moment when the exposure was completed; the level of triglycerides was taken as a lipid index for feeding-up status (cf. Tables 4 and 5). Neither ethanol nor toluene has influenced the level of lipid peroxidation in the serum, but the combined exposure to both significantly stimulated the formation of lipid peroxides in term of malondialdehyde by about 50% (Table 4). The level of triglycerides comprised in the normal, physiological range of 2.47 – 3.07 mmol/dm³. Xylene exerts a more profound effect as inferred from the increased formation of lipid peroxidation products both after single exposure (malondialdehyde ≈ 3.78 mmol/dm³) and combined exposure to xylene and ethanol (malondialdehyde ≈ 2.97 mmol/dm³) (Table 5). The level of triglycerides was still maintained within the physiological range (2.67 – 3.27 mmol/dm³).

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Table 4. Effects of single exposure of volunteers to ethanol and/or toluene on the level of triglycerides and lipid peroxidation in the serum

Groups	Number of subjects	Triglycerides (mmol/dm^3)	Lipid peroxidation (mmol MDA/dm^3)
Control	7	2.47 ± 0.17	2.04 ± 0.17
Ethanol	7	2.14 ± 0.25	1.97 ± 0.12
Toluene	7	2.42 ± 0.38	2.22 ± 0.25
Toluene + Ethanol	7	3.03 ± 0.33	2.53 ± 0.14^a

MDA – malondialdehyde.
Details as in Table 1. Results are the mean ± SE.
[a] – significantly different from the control at $p = 0.05$.

Table 5. Effect of single exposure of volunteers to ethanol and/or xylene on the level of triglycerides and lipid peroxidation in the serum

Groups	Number of subjects	Triglycerides (mmol/dm^3)	Lipid peroxidation (mmol MDA/dm^3)
Control	5	2.67 ± 0.28	2.28 ± 0.09
Ethanol	5	3.20 ± 0.38	2.70 ± 0.17
Xylene	5	3.06 ± 0.28	3.78 ± 0.20^c
Xylene + Ethanol	4	3.27 ± 0.62	2.97 ± 0.25^a

MDA – malondialdehyde. Details as in Tables 1 and 2. Results are the mean ± SE.
[a,c] – significantly different from the control at $p = 0.05$ and 0.001, respectively.

DISCUSSION

The biological monitoring of occupational exposure to the organic solvents, toluene and xylene, is generally based on the determination of hippuric and methylhippuric acid, respectively, in the urine and the environmental monitoring is guided by a current permissible limit value in air (20). The antipyrine test, used for monitoring the hepatotoxic and metabolic effects has been frequently applied in field studies of workers exposed to several chemicals, including solvents but there are no data reporting the combined effects of solvents and ethanol on antipyrine test in man yet.

The evaluation of the metabolic rate of the liver using the antipyrine test in the case of exposure to solvents superimposed on the ethanol ingestion was worth undetaking as combined exposures of volunteers to toluene/xylene and ethanol revealed that ethanol slowed down the elimination of the solvents from blood and decreased the excretion of the metabolites with urine (21, 23).

The study performed with volunteers, exposed to toluene (375 mg/m^3) and xylene (435 mg/m^3) singly or in combination with ethanol intake (steady blood level of ethanol $\approx$ 11 mmol/dm^3; former denomination $\approx$ 0.5%), revealed the lack of significant differences in salivary half-life ($T\ \frac{1}{2} \approx 12$ h) and clearance ($Cl_{AP} \approx \approx 0.83$ cm^3/s) between concurrent control and groups exposed to the solvents and/or ethanol. However, a tendency to increase the metabolic rate of antipyrine in xylene-exposed group ($T\ \frac{1}{2}$ 6.8 h; $Cl_{AP} \approx 1.40$ cm^3/s) and counteraction of the effect

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by ethanol (T $\frac{1}{2} \approx$ 15 h); $Cl_{AP} \approx 0.63$ cm^3/s) was noticed. The inhibitory effect of a higher dose of ethanol (blood level 37.8 mmol/dm^3 $\approx$ 1.72%) on the antipyrine metabolic rate was evident in an insubordinate volunteer exposed to xylene, where the clearance amounted to $Cl_{AP} \approx 0{,}20$ cm^3/s and half-life T $\frac{1}{2} \approx$ 42.3 h.

The analysis of the concentration of antipyrine in the saliva in the course of post-exposure period for reference control vs groups exposed to solvents and to solvents + ethanol revealed a statistically significant stimulating effect of xylene on metabolic rate of antipyrine (Table 3). The data seem to be unexpected as provoked by only one, 4-h exposure to xylene, however, they correspond with the general view, based on animal experiments, that xylene is a virtual inductor of hepatic monooxygenase system. The concentration of xylene found to induce cytochrome P-450 monooxygenase in rats comprises between 1200 – 12000 mg/m^3 with an exposure period of 1 – 4 weeks. The effect was assessed both *in vitro* on the basis of the activity of microsomal monooxygenase and *in vivo* directly by measuring the urinary excretion of methylhippuric acid and xylene-derived thioethers as well as indirectly by means of antipyrine test, where 24-h recovery of antipyrine and the metabolites in urine were determined (10, 18, 20, 30, 35). However, antipyrine salivary/plasma half-life or clearance values do not represent an absolute measure of an individual hepatic capacity to metabolize xenobiotics owing to the quantitative and qualitative heterogenicity of cytochrome P-450 monooxygenase system and their specific forms induced in response to either endogenous or exogenous inducers (15). It has been found that:

1. metabolism of antipyrine is catalyzed by different constitutive phenobarbital and 3-methylcholanthrene inducible isoenzymes of cytochrome P-450 (subfamilies IIB and IA, respectively) (10, 15);

2. aromatic organic solvents, toluene and xylene, represent phenobarbital-type inducers (18, 29);

3. ethanol induces a distinct cytochrome P-450 (subfamily IIE1) not involved in the metabolism of antipyrine but catalyzing the oxidation of the aromatics, toluene and xylene (18, 23).

It has been experimentally proved that the metabolic rate of antipyrine *in vivo* was stimulated after pretreatment of rats with xylene (29), phenobarbital (19), 3-methylcholanthrene (28) and ethanol as well (11). Thus, one may speculate that in the later instance, ethanol may act as a stimulator of the antipyrine metabolism qualitatively by modifying the membrane environment in the hepatic endoplasmic reticulum by allosteric effects or by displacing the other endogenous or exogenous substrate already bound to it (24).

In this study we reported that solvents-ethanol exposure of volunteers resulted in an increased level of lipid peroxides (TBARS – thiobarbituric acid reactive substances) in the serum. A commonly accepted view is that ethanol may contribute to the process of an enhanced formation of oxygen free radicals by cytochrome P-450 or by xanthine oxidase dependent reactions (25). This may be an important factor especially in alcoholics. One of the significant arguments in favour of this assumption was the finding in human alcoholics with hepatic cirrhosis the increased concentration of malondialdehyde and diene conjugates in the liver as well as pentane exhalation in comparison with the concentration found in non-alcoholics (26). Recent studies on rats have demonstrated that xylene, singly or combined with

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ethanol also exerts additive stimulatory effects on hepatic microsomal monooxygenases and enhanced lipid peroxidation rate (13, 35).

According to the current data, one may conclude that increased lipid peroxides in the serum would have some deleterious effects on intact tissues and organs. Numerous experimental details from the latest studies have been gathered to provide evidence for the etiopathogenetic role of lipid peroxidation in very diverse pathological processes, e.g. atherosclerosis, pathogenesis of cancer, inflammatory processes and toxic effects provoked by xenobiotics (cf. 37).

Summing up the human volunteer study: (1) there is a tendency to accelerate the metabolic rate of antipyrine, as an index of the activity of hepatic monooxygenase, under the influence of xylene, and (2) toluene and xylene exposure, superimposed on ethanol intake, provoke the increased formation of lipid peroxidation products in the serum. It may be suggested that the serum lipid peroxide level may be considered as a biological marker of effects of single and combined exposure to industrial organic solvents and additional extra factors e.g. ethanol.

ACKNOWLEDGEMENTS

The authors are very grateful to Ms. Helena Płatak, Ms. Wiesława Adamiak and Ms. Elżbieta Jabłońska for technical assistance and to Ms. Zofia Rudnicka for typing.
The study was done within a framework of the Polish Government Project CPBR 11.8.19 "Prevention of consequences of alcoholism and drug addiction".

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Received for publication: December 12, 1992
Accepted for publication: April 2, 1993

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DIARY OF CONFERENCES AND SEMINARS (contd)

June 8–11 1993, Kiev, Ukraine
XII Joint CIGR, IAAMRH, IUFRO International Symposium on Health and Ergonomic Aspects of Safe Use of Chemicals in Agriculture and Forestry
Info: Mrs Lydiya Yankovskaya, Symposium Coordinator, Dept. of Scientific and Medical Information, Inst. of Occupational Health, 75 Saksagansky St., 252033 Kiev, Ukraine
Tel. (44) 220 61 06, Fax (44) 220 66 77
Main topics:
* Occupational effects of harmful chemicals in Agriculture and Forestry, Permissible Levels,
* Health and ecological assessment criteria of new forms and methods of applicatinos of chemicals in agriculture and forestry,
* Occupational health services to farmers and farm workers dealing with pesticides,
* Health Surveillance Procedures,
* Ergonomics in design and operation of machines and equipment for application of chemicals in agriculture and forestry,
* Protective equipment, assessment of ergonomic aspects and efficiency,
* Methods of detoxification,
* Activity of international organizations on safe use of chemicals in agriculture and forestry.

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