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CYTOCHROME P–450 MEDIATED GENOTOXICITY OF TRIARYLMETHANE DYE IN MICE FED ETHANOL LIQUID DIET

TERESA WROŃSKA-NOFER[1], JUSTYNA WIŚNIEWSKA-KNYPL[1] and KALINA WYSZYŃSKA[2]

[1] Department of Toxiocological Biochemistry
[2] Department of Carcinogenesis
The Nofer Institute of Occupational Medicine
Lodz, Poland

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Abstract. Genotoxic effect of synthetic triarylmethane dye (Acid Green 16) was evaluated in Balb C mice fed nutritionally adequate liquid diet (1 kcal/ml) or isocaloric alcoholic diet containing 5% (w/v) ethanol (36% of total calories) for 6 days. Dye compound was given intraperitoneally at dose 150 mg/kg body wt. 30 h before test. The micronucleus test was used for evaluation of genotoxicity of the dye. The level of cytochrome P–450 and the activity of 7-ethoxycoumarin O-deethylase (ECOD) and 7-ethoxyresorufin O-deethylase (EROD) in microsomes were determined to assess the metabolic efficiency of the microsomal system. Acid Green 16 dye provoked an increased frequency of micronucleated polychromatic erythrocytes in bone marrow and ethanol enhanced this genotoxic effect through induction of cytochrome P–4502E1 and stimulation of activities of microsomal monooxygenases (ECOD and EROD), presumably catalyziung bioactivation of the dye.

INTRODUCTION

Epidemiological and clinical studies provide evidence that ethanol abuse is an important factor contributing to human cancer (12,13,22,32). It is worth noting that combined effect of ethanol ingestion and exposure to some potentially carcinogenic chemicals results in farther increase of cancer risk. Ethanol potentiates the carcinogenic effect of such chemicals as N-nitrozopyrrolidyne (15), dimethylhydrazine (26), N–nitrosamines and benzo(a)pyrene (8). Although ethanol alone does not lead to the development of tumors in experimental animals, it is reasonable to assume that alcohol consumption may be an important factor of chemically provoked carcinogenesis through stimulation of metabolic pathway for activation of procarcinogens/promutagens (11). Chemical carcinogens are usually chemically

Address reprint requests to Prof. T. Wrońska-Nofer, Department of Toxicological Biochemistry, The Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Lodz, Poland.

inert and require metabolic activation by cellular enzymes to exert their detrimental effect. Thus, chemical carcinogens need metabolic activation via cytochrome P−450 monooxygenases to generate reactive electrophiles capable of covalently binding to cellular macromolecules; the process may result in carcinogenic cell transformation owing to mutations in genes encoding enzymes or factors controlling cell division such as oncogenes or tumor suppressor genes (7). The prime mechanism through which ethanol mediates the conversion of procarcinogens to their reactive species is associated with stimulatory effect of ethanol on microsomal monooxygenases via induction of specific cytochrome P−4502E1 which catalyzes biotransformation of broad spectrum of industrial xenobiotics, thereby, more efficient transformation of procarcinogens/promutagens into ultimate carcinogenic/mutagenic forms (7). Stimulation of microsomal enzymes by ethanol accelerates metabolic activation of such genotoxic agents as aromatic amines (10), aliphatic dialkyl-nitrosamines (6) and polycyclic aromatic hydrocarbons e.g. benzo(a)pyrene (18).

The cytochrome P−450 monooxygenase systems generate superoxide, hydrogen peroxide, single oxygen and a hydroxyl-like radicals, which can participate in xenobiotics activation mechanism, oxidative DNA damage and chemical carcinogenesis (31).

Chronic ethanol administration induces cytochrome P−4502E1 (CYP2E1) and favours the microsomal free radical generation. Accumulation of active oxygen metabolites results in oxidative stress. Such oxidative stress is potentiated when various xenobiotics are administered together with ethanol. These effects appear to result from the unique capacity of the ethanol inducible CYP2E1 to activate xenobiotic agents via free radical mechanism (11, 34).

Triarylmethane dyes (e.g. Acid Green 16) are recognized as animal and human carcinogens (5). There is no evidence that would make clear whether carcinogenic/genotoxic effects of the compounds was produced by the dye itself or by its reactive, electrophilic metabolites. On the basis of chemical structure of the dye Acid Green 16, one may expect that the dye undergoes oxidative demethylation followed by reduction in microsomal enzyme system (20). Although the first step of the process represents the detoxification reaction, the further step leads to metabolic activation resulting in a more toxic derivative(s) (5). Moreover, in the course of biotransformation of the dye free radical species may be formed. Whether free radicals are involved in the cytotoxic effect of triarylmethane dyes remains to be elucidated.

In the present study it was assumed that ethanol preinduction may enhance the rate of biotransformation/bioactivation of an industrial triarylmethane dye, Acid Green 16, and in consequence its genotoxicity.

The aim of the study was: (1) to assess the genotoxic effect of triarylmethane dye, Acid Green 16, superimposed on a sustained ethanol intake in order to attain higher metabolic capacity of the liver via induction of microsomal monooxygenase system; (2) to find out whether the increased genotoxicity of the dye was related to inductive effect of ethanol on microsomal cytochrome P−450 monooxygenase system responsible for biotransformation of the dye to reactive, more toxic metabolites.

The study revealed high inductive potential of ethanol liquid diet on the cytochrome P−450 system and related enhancement of the genotoxic effect of Acid Green 16 dye in mice, probably via activation mechanisms.

MATERIALS AND METHODS

Animals and treatment

Male Balb C mice 9 weeks old (20–22 g) were acclimatized for 5 days in groups and fed laboratory chow Murigran and tap water *ad libitum*. Afterwards the mice were fed for 6 days either nutritionally adequate liquid diet prepared on the basis of Portagen (Polfa, licence of Mead Johnson BV) and containing 17% proteins, 22% fat, 54% carbohydrates, vitamins and trace elements or isocaloric ethanol liquid diet containing 5% (w/v) ethanol and representing 36% of total calories. The control diet and ethanol diet contained 1 kcal/ml corresponding to 4.2 kJ/ml (4,7). The mice consumed daily, on average, 21 g ethanol/kg body weight. In the morning (8.00 a.m.) on 5th day of feeding of liquid diet and isocaloric ethanol diet, the mice were given intraperitoneally either Acid Green 16 dye at dose 150 mg/kg body weight or physiological saline as reference control and sacrificied for test 30 h thereafter. The following experimental groups were analysed: control, ethanol, Acid Green 16, ethanol + Acid Green 16. As a reference two additional treatments were included: (1) Phenobarbital sodium – classic inducer of cytochrome P–450 (CYP2B6) monooxygenases – 0.05% water solution as the only drink for 9 days; (2) Mitomycin C – positive control for genotoxic effect – single intraperitoneal injection at dose 2.5 mg/kg body wt, 30 h before test.

Genotoxic effect was evaluated on the basis of micronucleus test (9). Metabolic capacity of the liver was assessed by determining the level of cytochrome P–450 and the activity of selected monooxygenases: (1) 7-ethoxycoumarin O-deethylase (ECOD) dependent on cytochrome P–450/448 – constitutively expressed as: CYP1A2, highly inducible by aryl hydrocarbons; CYP2B subfamily, highly inducible by phenobarbital class of compounds and CYP2B1 form by ethanol as well; CYP2E1, highly inducible by ethanol and a number of other small chemicals that are substrates for the enzyme; CYP3A subfamily, representing up to 60% of total P450 s, highly inducible by several xenobiotics; and all of the P–450s are involved in drug metabolism and procarcinogens activation, and (2) 7-ethoxyresorufin O-deethylase (EROD) dependent on cytochrome P–448 – constitutively not expressed as CYP1A1 and constitutively expressed as CYP1A2, both highly inducible by polycyclic aromatic hydrocarbons, capable of activating procarcinogens (7). ECOD and EROD were chosen for the present study because these monooxygenases could undergo stimulation by ethanol owing to induction of CYP2E1, CYP1A1, CYP2B1 and CYP3A1 (30).

Evaluation of the level of cytochrome P–450 and activities of microsomal monooxygenases

Mice were sacrificied by decapitation and livers were excised and homogenized with 8 volumes of KCl (150 mmol) buffered with Tris-HCl (10 mmol) pH 7.4 in a glass Potter-Elvehjem homogenizer with a teflon pestle to yield a 10% (w/v) homogenate. Microsomes were sedimented from 9 000 g postmitochondrial supernatant by ultracentrifugation at 4°C at 105 000 g for 1 h 15 min in Beckman XL-70 OPTIMA preparative ultracentrifuge, according to Dallner and Ernster (4). Microsomes, obtained from 7 ml of the 9 000 g postmitochondrial supernatants (≈ 0.85 g

of liver) were suspended by homogenization in 2.5 ml potassium phosphate buffer (0.1 mol) pH 7.4 and diluted directly before assay to a concentration 2 mg protein per ml of the suspension.

Protein in microsomes was determined with Folin phenol reagent by the method of Lowry et al. (14).

Cytochrome P−450 in the hepatic microsomes was determined according to Omura and Sato (19). Carbon monoxide difference spectra of dithionite-reduced microsomes were recorded between 490 and 450 nm using Beckman ACTA CIII spectrophotometer. An extinction coefficient of 91 $mmol^{-1}$ cm^{-1} was used for cytochrome P−450 quantifying.

Activity of 7-ethoxycoumarin O-deethylase (ECOD), was assayed according to Aitio (1).

Activity of 7-ethoxyresorufin O-deethylase (EROD), was determined according to Pohl and Fouts (21).

Ethanol in blood at the time of sacrificing of animals (2:00 p.m.) was determined enzymatically with alcohol dehydrogenase and NAD according to Bernt and Gutman (2).

Evaluation of genotoxicity

The micronucleus test was carried out on mice preinduced with ethanol and/or treated with single dose of Acid Green 16. Positive and negative reference controls received Mitomycin C or physiological saline, respectively. The bone marrow was sampled 30 h after the injections. The preparation of bone marrow and staining were carried out according to procedure described by Przybojewska et al. (23). The number of micronucleated cells in at least 1000 polychromatic erythrocytes and the ratio of polychromatic to normochromatic erythrocytes were determined for each animal.

Statistical analysis

The results were analysed statistically according to the Student t-test.

RESULTS

General effect of exposure

The weight gain of mice was slightly lower in the ethanol treated group in comparison with those consuming control liquid diet, probably due to ineffective utilization of calories derived from ethanol: the failure of ethanol energy conservation may be linked to increased microsomal oxidation of ethanol, a process which is not linked to ATP production. The mean daily ethanol intake amounted to 21 g/kg of body weight. Alcohol in blood, determined at the time of sacrificing of mice (after 6 days of feeding of ethanol liquid diet) was almost at the same level and amounted to 0.77 and 0.59% for ethanol and ethanol + Acid Green 16 groups, respectively (Table 1).

Table 1. The concentration of ethanol in blood of mice ingested of ethanol in liquid diet

Group	Ethanol concentration	
	‰	mmol/l
Control	0.04	0.9
Acid Green 16	0.03	0.7
Ethanol	0.77	16.7
Acid Green 16 + Ethanol	0.59	12.8

Ethanol 5% (w/v) in liquid diet or isocaloric liquid diet was given for 6 days. Blood for ethanol determination was punctured at the time of sacrificing.

Effect of ethanol and/or Acid Green 16 on the microsomal monooxygenases

Ethanol treatment resulted in a significant increase in hepatic microsomal protein and cytochrome P−450 content and associated monooxygenases activities. Cytochrome P−450 content increased 3-fold after 6 days of ethanol treatment. There was no significant difference in microsomal hepatic cytochrome P−450 levels between animals fed control liquid diet and those fed standard laboratory diet. It is worth noting that inductive potential of ethanol exceeds markedly that of phenobarbital (Table 2).

Table 2. The effect of chronic and short-term ethanol treatment and single dose of Acid Green 16 dye administration on microsomal monooxygenases activity in mice liver

Protocol of feeding	Group	Microsomal protein mg/g liver	Microsomal monooxygenases		
			Cyt. P−450 nmol/mg microsomal protein	ECOD pmol/min/mg microsomal protein	EROD
Liquid diet	Control	14.5 ± 0.36	0.55 ± 0.04	1320 ± 92	59 ± 4
	Acid Green 16	19.4 ± 0.33[c]	0.70 ± 0.03	1520 ± 77	57 ± 8
Ethanol liquid diet	Ethanol	23.8 ± 0.95[c]	1.82 ± 0.09[c]	11140 ± 670[c]	345 ± 16[c]
	Acid Green 16 + Ethanol	25.8 ± 0.91[c]	1.50 ± 0.08[c]	9060 ± 520[c]	262 ± 20[c]
Standard diet	Control	18.2 ± 0.44	0.65 ± 0.04	1705 ± 94	58 ± 5
	Phenobarbital (control-positive)	20.8 ± 0.66[c]	1.06 ± 0.06[cC]	6823 ± 241[cC]	184 ± 16[cC]

ECOD – 7-ethoxycoumarin O-deethylase; EROD – 7-ethoxyresorufin O-deethylase

Regimen of experiment:

Ethanol 5% (w/v) in liquid diet or isocaloric liquid diet was given for 6 days; Acid Green 16 (150 mg/kg) was given in a single dose, 30 h before testing.

Blood ethanol concentration at the time of animals sacrificing amounted to: 0.6−0.8‰ (mice metabolized ethanol 3−5 times faster than human).

Values are the mean ± SE for 6−8 mice

[c]significantly different from the control group, $p = 0.05$, at least

[C]significantly different from ethanol groups

ECOD and EROD activities after 6 days of ethanol liquid diet feeding was elevated 8.4-fold and 5.7-fold, respectively, in comparison with controls. Administration of Acid Green 16 to mice pretreated with ethanol liquid diet resulted in a slight reduction of inductive effect of ethanol itself. Acid Green 16 administered singly at dose 150 mg/g body wt affected neither the level of cytochrome P−450 nor the activities of examined enzymes ECOD and EROD (Table 2).

Effect of ethanol on genotoxic effect of Acid Green 16

Acid Green 16 caused a significant and dose related increase in the frequency of polychromatic erythrocytes with micronuclei in the bone marrow of mice (Table 3).

Table 3. The frequency of polychromatic erythrocytes with micronuclei in bone marrow of mice in dependence to the dose of Acid Green 16

Genotoxic substance		Polychromatic erythrocytes with micronuclei	Erythrocytes ratio polychrom./normochrom.
Group	Total dose (mg/kg)	Frequency (%)	P/N
Untreated (control)	0	0.2 ± 0.00	1.27
Acid Green 16	70	0.6 ± 0.13[c]	1.12
	90	0.6 ± 0.20[c]	1.17
	180	0.8 ± 0.21[c]	1.13
	360	1.3 ± 0.25[c]	0.82[c]
Mitomycin C (control-positive)	2.5	3.9 ± 0.75[c]	0.41[c]

Acid Green 16 was given intraperitoneally, in a single dose, 30 hours before testing. Animals were fed standard laboratory chow Murigran and tap water *ad libitum*.
Values are the mean ± SE for 6−8 mice.
[c]significantly different from the control group, p = 0.05, at least.

After a single injection of Acid Green 16 at dose 150 mg/kg body wt, the number of micronucleated polychromatic erythrocytes increased 4-fold over that of controls.

Table 4. Enhancement of genotoxic effect of Acid Green 16 by ethanol ingestion in mice as assayed by the bone marrow micronucleus test

Protocol of feeding	Groups	Polychromatic erythrocytes with micronuclei	Erythrocytes ratio polychrom./normochrom.
		Frequency (%)	P/N
Liquid diet	Control	0.22 ± 0.04	0.96
	Acid Green 16	0.80 ± 0.06[c]	0.93
Ethanol liquid diet	Ethanol	0.32 ± 0.04[c]	0.79
	Acid Green 16 + Ethanol	1.10 ± 0.09[c]	0.49[c]
Standard diet	Mitomycin C (control-positive)	3.90 ± 0.75[c]	0.41[c]

Regimen of experiment as described under Table 2.
Values are the mean ± SE for 6-8 mice.
[c]significantly different from the control group, p = 0.05, at least.

Ethanol treatment had no effect on the spontaneous frequency of polychromatic erythrocytes with micronuclei. However, inductive effect of ethanol on the cytochrome P−450 system (Table 2) resulted in enhanced activation of Acid Green 16 to genotoxic form. The number of micronucleated polychromatic erythrocytes in the bone marrow of mice fed ethanol liquid diet and singly treated with Acid Green 16 was ≈ 40% higher than in mice treated with Acid Green 16 only (Table 4).

DISCUSSION

Many known genotoxic agents fall into the category of chemicals that need metabolic activation. Lack of metabolic activation has been one of the major limitations of studies *in vitro*. Furthermore, the activation process in studies *in vivo* may facilitate a rapid screening of substances for their genotoxic potential. Data from such a study would be of value for setting priorites for more definite testing of the metabolic pathway and genotoxic species of the compounds in question. The genotoxic potential of Acid Green 16 superimposed on ethanol preinduction may be the case.

In the present study a short-term model of alcoholization of mice, employing ethanol liquid diet to achieve induction of hepatic monooxygenases which metabolize both, xenobiotics and ethanol, was applied successfully. It was aimed at elucidating whether a stimulatory effect of ethanol on the enzymes, owing to predominant induction of cytochrome P−4502E1, enhances the genotoxic effect of Acid Green 16, via metabolic activation in this system. The induction of microsomal monooxygenase by ethanol was first shown in rat by Rubin and Lieber (24). Since that time, many studies have confirmed the effect in rats (25,26,33), rabbits (17), mice (27), hamsters (10,15) and men (24). It was also indicated that the extent of the cytochrome P−450 enzymes induction is related to the exposure regimen, dosage, route and duration of ethanol administration, e.g. in drinking water (33,35), in liquid diet (27) or vapour inhalation (16).

The results of this study show that after 6 days of ethanol administration in liquid diet, the mouse hepatic cytochrome P−450 (CYP2E1) level increased by three times when compared with the control and metabolic capacity of the system, as expressed by ECOD and EROD, raised many more times (Table 2). As inferred from the data, both assayed enzymes, ECOD (preferentially) and EROD (to minor extent) responded to ethanol induction of CYP2E1. Any of the substrates tested, namely neither 7-ethoxycoumarin nor 7-ethoxyresorufin, was specific for CYP2E1. However, 7-ethoxycoumarin was indicated in the study to be a satisfactory substrate of CYP2E1 to demonstrate the induction capacity of ethanol (and phenobarbital, as reference). The 7-ethoxyresorufin substrate, more specific for CYP1A subfamily, was also metabolized at higher rate in the system of CYP2E1. The above results may be related to cross-reactivity of CYP2E1 with reaction catalyzed by CYP2A1/2 and ethanol-stabilized cytochrome P−450s protein. A significant increase in hepatic cytochrome P−450 with concomitant increase of microsomal protein content in ethanol treated mice (Table 2) confirmed that the effect induced by ethanol on the microsomal monooxygenase system depends on substrate (ethanol) − mediated cytochrome P−450 protein stabilization in 2E1 form (7,28,29). The ubiquitous nature of CYP2E1 substrates in the industrial workplaces and environment has marked human health implications. In the case of CYP2E1 induction owing to

ethanol abuse many industrial chemicals can be metabolized with a higher rate resulting in alteration of potential health hazard from combined exposure. The extent of induction of the CYP2E1 dependent monooxygenases by ethanol seems to be high enough to increase the toxic/genotoxic potential of some agents through the enhancement of metabolic activation: the increased frequency of micronucleated polychromatic erythrocytes provoked by Acid Green 16 in ethanol-preinduced mice carroborates this assumption (Table 4).

The results of this study suggest biotransformation of Acid Green 16 via CYP2E1-dependent bioactivation as inferred from its increased genotoxicity in ethanol-preinduced mice. Enhancement of genotoxic effect of the dye by ethanol is closely related to stimulatory effect of ethanol on cytochrome P-4502E1-dependent microsomal monooxygenases in the liver. Treatment of mice with ethanol, especially effective in a form of ethanol liquid diet, led to the induction of CYP2E1 enzymatic protein in the liver as manifested by a pronounced increase in the level of cytochrome P−450, (CYP2E1), the content of microsomal protein and metabolic rate of associated enzymes, ECOD and EROD (Table 2). In a final analysis, an enhancement of frequency of polychromatic erythrocytes with micronuclei in the bone marrow of mice jointly exposed to Acid Green 16 dye and ethanol may be taken as an end point of genotoxic effect of the arylmethane dye (industrial hazard factor) presumably related to CYP2E1-dependent activation pathway induced by ethanol (environmental extra-factor).

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REFERENCES

1. Aitio A. A simple and sensitive assay of 7-ethoxycoumarin deethylation. Analyt Biochem 85: 488−491, 1978.
2. Bernt E, Gutman I. Ethanol determination with alcohol dehydrogenase and NAD. In: Methods on Enzymatic Analysis, ed. HU Bergmayer, vol. 3, Verlag Chemie, Weinheim, Academic Press, New York, 1449−1502, 1974.
3. Dallner G, Ernster L. Subfractionation and comparison of microsomal membranes: a review. J Histochem Cytochem 16: 611−632, 1968.
4. DeCarli LM, Lieber CS. Fatty liver in the rat after prolonged intake of ethanol with a nutritionally adequate liquid diet. J Nutr 91: 331−336, 1967.
5. Docampo R, Moreno SV. The metabolism and mode of action of gentian violet. Drug Metab Rev 22 (2−3): 161−178, 1990.
6. Garro AJ, Seitz HK, Lieber CS. Enhancement of dimethylnitrosamine metabolism and activation to a mutagen following chronic ethanol consumption. Cancer Res 41: 120−124, 1981.
7. Gonzalez FJ, Gelboin HV. Role of human cytochromes P−450 in the metabolic activation of chemical carcinogens and toxins. Drug Metab Rev 26: 165−183, 1994.
8. Griciute L, Monceviciute-Eringiene E, Characieus D, Domkiene V, Barauskaite S. Influence of ethanol on some host reactions in the early stages of N-nitrosamine carcinogenesis. Modul. Exp. Carcinogenesis IARC Sci Publ 51: 197−203, 1983.
9. Heddle JA, Stuart E, Salamone MF. The bone marrow micronucleus test. In: Handbook of Mutagenicity Test Procedures, ed. BJ Kilbey, W Legator, W Nichols, C Ramel, Elsevier Sci Publ BV, 441−457, 1984.

10. Ioannides C, Steele C. Hepatic microsomal mixed-function oxidase activity in ethanol-treated hamsters and its consequences on the bioactivation of aromatic amines to mutagens. Chem Biol Interact 59: 129–139, 1986.
11. Lieber CS. Interaction of ethanol with drug hepatotoxic agents, carcinogens and vitamins. Alcohol, Alcoholism 25: 157–176, 1990.
12. Lieber CS, Seitz HK, Garro AJ, Warner TM. Alcohol-related diseases and carcinogenesis. Cancer Res 39: 2863–2867, 1979.
13. Lowenfels AB. Alcohol and cancer. NY State J Med 74: 56–59, 1974.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275, 1951.
15. McCoy G, Hecht S, Furuya K. The effect of chronic ethanol consumption on the tumorigenicity of N-nitrosopyrrolidyne in male Syrian golden hamsters. Cancer Lett. 33: 151–159, 1986.
16. Morgan ET, Devine M, Skett D. Changes in the rat hepatic mixed function oxidase system associated with chronic ethanol vapor inhalation. Biochem. Pharmacol. 30: 595–601, 1981.
17. Morgan ET, Koop DR, Coon MJ. Catalytic activity of cytochrome P–450 isozyme 3a from liver microsomes of ethanol-treated rabbits. J Biol Chem 257: 13951–13955, 1982.
18. Neis J, Brommelstroet B, van Gemert P, Roelofs H, Henderson P. Influence of ethanol agents by induction on the metabolic activation of genotoxic agents by isolated rat hepatocytes. Arch Toxicol 57: 217–221, 1985.
19. Omura T, Sato R. The The carbon monoxide-binding pigment of liver microsomes. Evidence for its hemoprotein nature. J Biol Chem 239: 2370–2378, 1964.
20. Parke DV. The Biochemistry of Foreign Compounds, Pergamon Press, Oxford, London, Edinburgh, New York, 1968.
21. Pohl RJ, Fouts JR. A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. Analyt Biochem 107: 150–155, 1980.
22. Pollack ES, Nomura AMY, Heilbrum LK, Stemmermann GN, Green SE. Prospective study of alcohol consumption and cancer. N Engl J Med 310: 617–621, 1984.
23. Przybojewska B, Barański B, Spiechowicz E, Szymczak W. Mutagenic and genotoxic activity of chosen dyes and surface active compounds used in the textile industry. Pol J Occup Med 2: 171–185, 1989.
24. Rubin E, Lieber CS. Hepatic microsomal enzymes in man and in rat: induction and inhibition by ethanol. Science 162: 690–691, 1968.
25. Rush GF, Adler VL, Hook JB. The effect of ethanol administration on renal and hepatic mixed function oxidases in the Fisher 344 rat. Toxicol Lett 12: 265–270, 1982.
26. Seitz H, Czygan P, Waldherr R, Veith S, Raedsch R, Kossmodel B. Enhancement of 1,2-dimethylhydrazine-induced rectal carcinogenesis following chronic ethanol consumption in the rat. Gastroenterology 86: 886–891, 1984.
27. Schnellmann RG, Wiersma DA, Randall DJ, Smith TL, Sipes IG. Hepatic mixed function oxygenase activity and glutatione S-transferase activity in mice following ethanol consumption and withdrawal. Toxicology 32: 105–116, 1984.
28. Song BJ, Gelboin HV, Park SS, Yang CS, Gonzalez FJ. Complementary cDNA and protein sequence of ethanol – inducible rat and human cytochromes P–450s: transcriptional and post-transcriptional regulation of the rat enzyme. J Biol Chem 261: 16689–16697, 1986.
29. Song BJ, Veech RL, Park SS, Gelboin HV, Gonzalez FJ. Induction of rat hepatic N-nitrosodimethylamine demethylase is due to protein stabilization. J Biol Chem 264: 3568–3572, 1989.
30. Soucek P, Gut I. Cytochromes P–450 in rats: structures, functions, properties and relevant human forms. Xenobiotica 22: 83–103, 1992.
31. Trush MT, Kensler TW. Role of free radical in carcinogen activation. In: Oxidative Stress, ed. H Sies, Academic Press, Lonon, 421–443, 1991.
32. Tuyns AJ. Epidemiologiy of alcohol and cancer. Cancer Res. 39: 2840–2843, 1979.
33. Wiśniewska-Knypl JM, Wrońska-Nofer T, Jajte J, Jedlińska U. The effect of combined exposure to ethanol and xylene on rat hepatic microsomal monooxygenase activities. Alcohol 6: 347–352, 1989.

34. Wiśniewska-Knypl JM, Wrońska-Nofer T. Biological markers of oxidative stress induced by ethanol and iron overload in rat. Int J Occup Med Environ Health 7: 355–363, 1994.
35. Zawadzka M, Barański B, Wiśniewska-Knypl JM, Wrońska-Nofer T. Genotoxic effect of triarylmethane dye–Acid Green 16 after chronic ethanol consumption in mice. Pol J Occup Med Environ Health 6: 391–397, 1993.

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DIARY OF CONFERENCES AND SEMINARS (contd)

The symposium will continue the International Symposium on Biological Monitoring of the International Commission on Occupational Health (ICOH) in 1992 in Kyoto, Japan. Symposium will be organized by the Finnish Institute of Occupational Health, and it is a satellite symposium to the ICOH Congress in Stockholm on 15-20 September 1996.
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