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Effect of ethanol on the urinary excretion of cyclohexanol and cyclohexanediols, biomarkers of the exposure to cyclohexanone, cyclohexane and cyclohexanol in humans

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Objectives This study explored the acute effect of ethanol (EtOH) on the urinary excretion of cyclohexanol (CH-ol), 1,2- and 1,4-cyclohexanediol (CH-diol), biomarkers of exposure to important solvents, and chemical intermediates cyclohexanone (CH-one), cyclohexane (CH) and cyclohexanol.

Methods Volunteers (5—8 in each group) were exposed for 8 hours either to CH-one, CH or CH-ol vapor at concentrations of about 200, 1000, and 200 mg/m^3, respectively, with concomitant ingestion of EtOH (4 14-g doses taken during the exposure). Urine was collected for 72 hours and analyzed for CH-ol and CH-diols using a procedure involving acidic hydrolysis and gas chromatographic determination.

Results The metabolic yields of CH-ol, 1,2-, and 1,4-CH-diol, respectively, in the exposures with EtOH were as follows: 11.3%, 36%, 23% after the exposure to CH-one, 3.1%, 15%, 8% after the exposure to CH, and 6.6%, 24%, 18% after the exposure to CH-ol. [The corresponding values obtained previously in matching experiments without EtOH were as follows: 1.0%, 39%, 18% (CH-one); 0.5%, 23%, 11% (CH); and 1.1%, 19%, 8% (CH-ol).] The excretion curves of the metabolites in the exposures with EtOH were not delayed when compared with the corresponding curves of a comparison group.

Conclusions The urinary excretion of CH-diols is much less sensitive to EtOH than that of CH-ol. It is recommended to employ CH-diols as useful and more reliable biomarkers of exposure to CH-one, CH and CH-ol in field examinations.

Key terms biological monitoring, metabolic interference.

In our previous papers on the human metabolism of the important solvents and chemical intermediates cyclohexanone (CH-one), cyclohexane (CH) and cyclohexanol (CH-ol), we demonstrated that 1,2- and 1,4-cyclohexanediol (CH-diol) are major urinary metabolites and useful new biomarkers of exposure to each of the title compounds (1, 2). Hitherto, biomonitoring of CH-one and CH has been based solely on the determination of a minor metabolite, CH-ol (3—5).

Acute ethanol (EtOH)-induced effects on the metabolism of other xenobiotics, based on the inhibition of alcohol dehydrogenase (ADH) or the microsomal oxidative system P450 involved in the biotransformation of numerous organic compounds, is a well known phenomenon. The consumption of alcoholic beverages shortly before or during a workshift (or in model laboratory experiments) modifies urinary levels of metabolites of styrene (6), xylenes (7), trichloroethylene (8) and other solvents significantly. As to CH-one, CH, or CH-ol, the reduction of CH-one to CH-ol (the primary step in the metabolism of CH-one) is catalyzed by ADH (9), while the oxidation of CH to CH-ol (the primary step in the metabolism of CH) is mediated by the P450 system enzymes (10). The enzymes responsible for the subsequent oxidation of CH-ol to CH-diols have not, as yet, been identified, but they are likely to belong to the P450 enzymes, too. Thus interference of EtOH with the metabolism of CH-one, CH, and CH-ol can be anticipated.

In this study, the effect of EtOH on the urinary excretion of CH-ol and CH-diols following exposures to

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CH-one, CH, or CH-ol vapor was evaluated for human volunteers.

Subjects and methods

General design

The experiments and methods employed were basically those used in our previous studies (1, 2). Volunteers were exposed under defined conditions either to CH-one, CH, or CH-ol vapor at concentrations close to the respective occupational exposure limits with concomitant ingestion of EtOH. Their urine was collected and analyzed for CH-ol, 1,2-, and 1,4-CH-diol. The results were compared with those obtained previously in matching experiments without EtOH.

Subjects

Four women and 5 men, aged 32 to 54 years, volunteered to participate in the experiments. Most of them took part in the previous investigations (1, 2), from which the reference data used in this study were obtained. The scheme of participation of the subjects in the exposure experiments is shown in table 1.

All the subjects were healthy and showed no abnormalities in routine clinical examinations, none of them admitted having an excessive drinking habit or drug abuse, and none of them drank alcohol (except for the defined experimental doses) or took medicine 1 day before or during the collection of urine. The experiments were conducted with informed consent according to the recommendations of the Declaration of Helsinki (11) and were approved by the Scientific Committee of the Czech Ministry of Health.

Table 1. Scheme of participation of the subjects in the exposure experiments. (CH-one = cyclohexanone, CH = cyclohexane, CH-ol = cyclohexanol, EtOH = ethanol, + = participation)

Subject	Gender	Exposure					
		CH-one		CH		CH-ol	
		Without[a] EtOH	With EtOH	Without[b] EtOH	With EtOH	Without[b] EtOH	With EtOH
1	Female	+	+	+	+	+	+
2	Female	+	+	+	+	+	+
3	Female	+	+	+	+	+	+
4	Female	+	+	+	+	+	+
5	Male	+	+	+	+	+	+
6	Male	+	+	+		+	
7	Male		+	+		+	+
8	Male		+	+		+	+
9	Male	+					
10	Male	+					
11	Male						+

[a] Control, carried out previously and described in reference 1.
[b] Control, carried out previously and described in reference 2.

Exposures

The exposures to the vapor of the CH compounds were conducted with groups of 2 to 4 subjects in a closed exposure chamber (volume, 64 m^3) with automatic maintenance of the vapor concentration, as described previously (1, 2). In brief, the calculated amounts of liquid CH-one, CH, or CH-ol were evaporated before the exposure, and further vapor supply was controlled by feedback from a Carlo Erba gas chromatograph equipped with an automatic gas-sampling valve, analyzing the atmosphere at 5-minute intervals. The analyses were performed on a stainless steel column (0.5 m × 5 mm) filled with 20% Carbowax 20M on silanized Gas Chrom P and operating at 50°C (CH) or 100°C (CH-one, CH-ol). The coefficient of variation of the concentration measurements during the exposures ranged from 4% to 9%. The mean temperature and relative humidity during the exposures were 26°C and 65%, respectively.

All the exposures were conducted over a period of 8 hours (from 0800 to 1600).

EtOH, 4 doses, each 14 g (=18 ml) (corresponding to the EtOH content of 0.5 l of weak beer), diluted with orange juice, was ingested at 0900, 1100, 1300, and 1500 during the inhalation exposures or at 1600, 1800, 2000, and 2200 after the CH-one exposure. The subjects were at rest during the exposures. They left the exposure chamber at 2-hour intervals for 2—3 minutes to void urine.

The exposures with EtOH were performed within 12 months after the matching control experiments.

Measurement of pulmonary ventilation and retention in the respiratory tract

Minute respiratory volume was measured by Wright respirometers 12 times at 10-minute intervals, regularly spread throughout the exposure. Mean retention in the respiratory tract (r) was calculated from the vapor concentration in exhaled (C_e) and inhaled (C_i) air: r (%) = 100 (1- C_e/C_i). The analysis of the mean exhaled air, which was conducted 4 times throughout the exposure (in about 1, 3, 5 and 7 h) for each subject, consisted of forcing the breath through a metal tube into a 7-l silanized glass vessel, where it was passively mixed and then withdrawn by continuous suction into a manually operated sampling valve of the gas chromatograph. Inhaled ambient air was measured simultaneously using an automatic sampling valve.

Analysis of the urine

Urine (18 samples per person per experiment, see figures 1—3) was collected for 72 hours and kept frozen until the analysis. CH-ol, 1,2-, and 1,4-CH-diol were determined by the gas chromatographic method of Flek & Šedivec (1, 12). The standard procedure involved acidic hydrolysis of the glucuronide conjugates. Therefore, each

determined compound represented the sum of its conjugated and unconjugated forms.

Results

The ingestion of EtOH in 4 doses, 14 g each, during 8-hour inhalation exposures to CH-one, CH, and CH-ol at concentrations close to the occupational exposure limits (200, 1050 and 200 mg/m^3, respectively) interfered with the metabolism of these compounds. The most striking effect was a several-fold increase in the yield of urinary CH-ol as compared with the exposures without EtOH (expressed as the percentage of the absorbed dose of the parent compound), from 1.0% to 11.3% (CH-one), from 0.5% to 3.1% (CH), and from 1.1% to 6.6% (CH-ol), respectively. The metabolic yields of CH-diols were affected by EtOH to a much smaller extent, being almost unchanged in the case of CH-one, decreased in the case of CH, and increased in the case of CH-ol (table 2).

The excretion curves of CH-ol and CH-diols exhibited no EtOH-induced time shift of their maximums; nevertheless, the plots of 1,2-CH-diol appeared to be slightly flattened in the exposures with EtOH (figures 1—3).

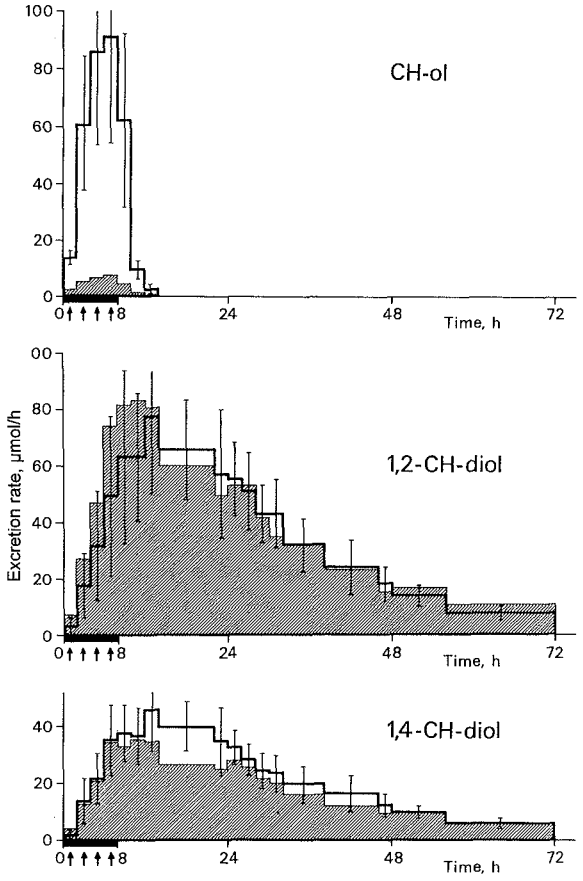

Figure 1. Urinary excretion of cyclohexanol (CH-ol), 1,2-cyclohexanediol (1,2-CH-diol), and 1,4-cyclohexanediol (1,4-CH-diol) after a single 8-hour exposure to cyclohexanone (CH-one) vapor (189 mg/m^3) with concomitant ingestion of 4 14-g doses of ethanol (EtOH) (arrows). Hatched diagram is for the control exposure to CH-one, 207 mg/m^3, carried out previously (1). Bars indicate the standard deviations for 8 subjects (shown only for the exposure with EtOH).

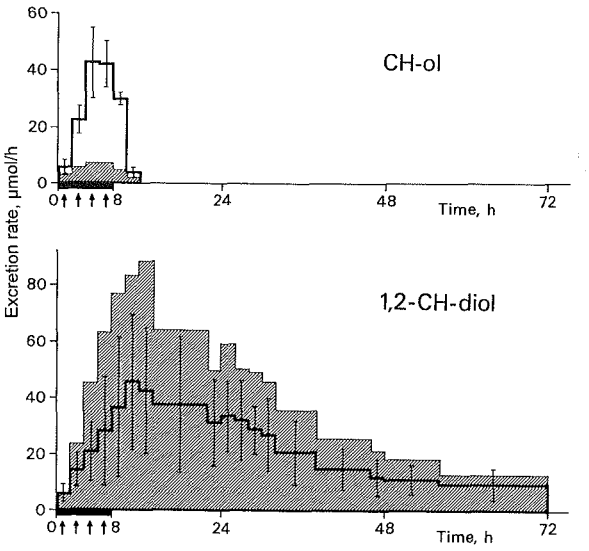

Figure 2. Urinary excretion of cyclohexanol (CH-ol) and 1,2-cyclohexanediol (1,2–CH-diol) after a single 8-hour exposure to cyclohexane (CH) vapor (1005 mg/m^3) with concomitant ingestion of 4 14-g doses of ethanol (EtOH) (arrows). The hatched diagram is for the control exposure to CH, 1010 mg/m^3, carried out previously (2). Bars indicate the standard deviations for 5 subjects (shown only for the exposure with EtOH).

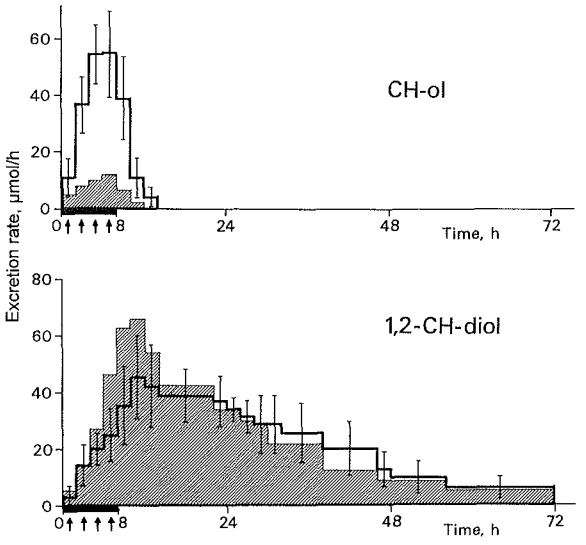

Figure 3. Urinary excretion of cyclohexanol (CH-ol) and 1,2-cyclohexanediol (1,2-CH-diol) after a single 8-hour exposure to CH-ol vapor (186 mg/m^3) after concomitant ingestion of 4 14-g doses of ethanol (EtOH) (arrows). The hatched diagram is for the control exposure to CH-ol, 236 mg/m^3, carried out previously (2). Bars indicate the standard deviations for 8 subjects (shown only for the exposure with EtOH).

Table 2 . Mass balance of cyclohexanone (CH-one), cyclohexane (CH) and cyclohexanol (CH-ol) in humans after 8 hours of inhalation exposure with concomitant ingestion of 4 14-g doses of ethanol (EtOH). The data in italics are from control exposures carried out previously. (1,2-CH-diol =1,2-cyclohexanediol, 1,4-CH-diol = 1,4-cyclohexanediol)

Compound(s)	N	Concentration in air (mg/m³)		Minute respiratory volume (l/min)		Retention in respiration tract (%)		Total absorbed dose[a] (mmol)		Proportion (%) of the dose excreted in urine during 72 h					
										CH-ol		1,2-CH-diol		1,4-CH-diol	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CH-one															
With EtOH	8	189	17	11.5	2.3	58.3	3.6	6.11	1.12	11.3	3.3	35.9	6.0	22.9	2.5
Without EtOH[b]	8	207	3	11.0	2.3	58.1	1.3	6.42	1.20	1.0	0.3	38.5	5.3	18.2	2.1
CH															
With EtOH	5	1005		11.9	2.1	14.2	4.4	9.4	2.0	3.1	0.9	15.2	5.1	7.9	1.9
Without EtOH[c]	8	1010	35	10.8	2.5	18.4	3.9	11.1	1.0	0.5	0.2	23.4	4.2	11.3	2.9
CH-ol															
With EtOH	8	186	5	11.5	1.3	63.8	2.3	6.50	0.62	6.6	1.2	24.1	4.9	18.0	2.9
Without EtOH[c]	8	236	2	11.7	2.5	64.2	1.9	8.46	1.85	1.1	0.3	19.1	3.8	8.4	1.4

[a] Calculated as a product of the concentration of the CH compound in the air, pulmonary ventilation during 8 hours, and retention in the respiratory tract.
[b] Control, data from reference 1.
[c] Control, data from reference 2.

The uptake of the parent CH compounds in the matching exposures with and without EtOH was not exactly the same (table 2). Therefore, the effect of EtOH on the excretion rate of CH-ol and CH-diols was not quantified by direct comparison of the values from matching experiments as plotted in figures 1—3; instead a correction for differences in the uptake had to be included. Thus the comparison was made between excretion rates obtained in the experiments with and without EtOH, the latter being multiplied by the ratio of uptakes in the exposures with and without EtOH (table 3).

The ingestion of EtOH (4 14-g doses) during a 6-hour period after the inhalation exposure to CH-one resulted in a several-fold rise of urinary CH-ol (figure 4), while no significant effect on the level of CH-diols was observed (not shown).

Table 3 Relative excretion rate of cyclohexanol (CH-ol) and cyclohexanediols (CH-diol) in the last 2-hour period of 8-hour inhalation exposures to cyclohexanone (CH-one), cyclohexane (CH) and CH-ol with concomitant ingestion of 4 14-g doses of ethanol (EtOH).

Compounds[a]	Relative excretion rate[b] (%)					
	CH-ol		1,2-CH-diol		1,4-CH-diol	
	Mean	SD	Mean	SD	Mean	SD
CH-one+EtOH	1270	520	70	39	108	38
CH+EtOH	750	140	52	35	52	32
CH-ol+EtOH	620	170	69	25	140	57

[a] CH-one, 189 mg/m³; CH, 1010 mg/m³; CH-ol, 186 mg/m³; EtOH, 4 14-g doses taken 1, 3, 5 and 7 hours after the start of the exposures to CH compounds.
[b] The relative excretion rates were calculated as the ratio of the corresponding excretion rates in matching experiments with and without EtOH, respectively, after correction for different uptakes of the parent CH compounds in the experiments. The excretion rates in the control experiments were designated as 100%.

Discussion

Numerous examples have been reported on the metabolic interactions of various xenobiotics with EtOH. The most common mechanism is the competition between EtOH and xenobiotics for ADH or the microsomal P450 oxidation system. Inhibition of the primary metabolic reaction produces an elevated blood level of the parent compound, paralleled by reduced excretion of the metabolites. The excretion rates of the metabolites can attain levels characteristic for control experiments after the elimination of EtOH from the organism, the excretion

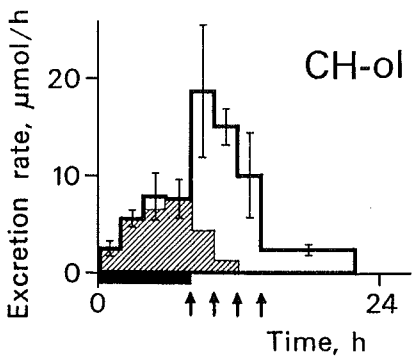

Figure 4. Urinary excretion of cyclohexanol (CH-ol) after a single 8-hour exposure to cyclohexanone (CH-one) vapor (185 mg/m³) with subsequent administration of 4 14-g doses of ethanol (EtOH) (arrows). The hatched diagram is for the control exposure to CH-one, 207 mg/m³, carried out previously (1). Bars indicate the standard deviations for 4 subjects (shown only for the exposure with EtOH).

peaks then being shifted for up to several hours. Similarly, inhibition occurring at a stage of a metabolic intermediate results in an accumulation of the intermediate in blood and increased elimination of this intermediate in urine. In some cases, the inhibitory effect of EtOH has been attributed not to competition for an active site of an enzyme but instead to competition for the cofactor NAD+ (nicotinamide adonine dinucleotide), which was depleted due to involvement in the ADH-mediated oxidation of EtOH (13). On the other hand, the increased concentration of the ADH-NADH complex, produced in the metabolism of EtOH, accounted for the EtOH-accelerated reduction of chloral hydrate (14) or CH-one (13), mediated by ADH and requiring NADH (reduced form of nicotinamide adenine dinucleotide). Involvement of this mechanism was supported by the finding of the transfer of hydrogen between CH-one and EtOH after their simultaneous administration (9). In vivo, faster decay of CH-one and a faster rise of CH-ol in blood were observed after the coadministration of EtOH and CH-one to rabbits (13).

In our present study, coadministration of EtOH with CH-one, CH, or CH-ol modified the metabolism of all the parent compounds in a similar manner. The uniform several-fold increase in urinary CH-ol indicates that inhibition occurs at a metabolic step common to each of the title compounds (ie, oxidation of CHol). On the other hand, the overall production of CH-diols was not affected by EtOH as dramatically, perhaps due to compensation of the inhibition by an elevation of the blood level of CH-ol. The observed slight differences in the effect of EtOH on the metabolism of each particular CH compound are difficult to interpret unless more detailed knowledge on the kinetics of relevant partial reactions becomes available. The aforementioned reactions include (i) oxidation of CH to CH-ol, (ii) reduction of CH-one to CH-ol, (iii) oxidation of CH-ol to CH-diols, and (iv) side reactions. The occurrence of the last one is only deduced from the finding that the amounts of excreted CH-ol and CH-diols accounted for not more than 30% to 60% of the absorbed parent compounds. There is also an indication that CH-diols undergo reactions other than the conjugations since, after ingestion of 1,2- and 1,4-CH-diol, only 80% and 60% of the doses, respectively, were excreted in free and conjugated forms (2).

Regardless of the detailed mechanism of metabolic interactions between CH-compounds and EtOH, practical considerations can be drawn on the basis of the obtained results. While several authors suggested employing CH-ol as a biomarker of the exposure to CH and CH-one (3—5), we propose using CH-diols alternatively or complementarily to CH-ol. Our observation that the excretion of urinary CH-diols is affected by EtOH much less than that of CH-ol supports preferential use of CH-diols as more reliable biomarkers of the exposure to CH, CH-one and CH-ol, especially in workplaces where alcoholic beverages are likely to be drunk.

Acknowledgments

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