

Occupational exposure to solvent mixtures: effects on health and metabolism

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Abstract

Exposure monitoring by personal diffusive samplers, biological monitoring of toluene exposure by urinary hippuric acid determination, haematology, serum biochemistry for liver function, and a subjective symptom survey by questionnaire were conducted on 303 male solvent workers. They were exposed to a mixture of solvents including toluene (geometric mean 18 ppm), methyl ethyl ketone (MEK; 16 ppm), isopropyl alcohol (IPA; 7 ppm), and ethyl acetate (9 ppm). The intensity was mostly below unity using the additiveness formula based on current Japanese occupational exposure limits, but more than eight times unity at the maximum. The results were compared with the findings in 135 non-exposed male workers of similar ages. Haematology and liver function tests did not show any exposure related abnormality, and subjective symptoms were mostly related to central nervous system depression and local irritation. Further analysis suggested that the irritation effects were not related to exposure to MEK. Analysis of the relation between toluene exposure and hippuric acid excretion in urine showed that there was no metabolic interaction between MEK and toluene, or between IPA and toluene. Overall, therefore, it is concluded that there was no sign or symptom detected to suggest anything other than toluene toxicity, that there was no evidence to indicate any modification of toluene toxicity or metabolism due to coexposure, and that the additiveness assumption is reasonable for risk assessment for the combination of solvents under these exposure conditions.

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Organic solvents are common materials in modern industries. They are generally used as mixtures, typically in thinner, adhesive, ink, and paint preparation.¹⁻³ This contrasts with the fact that the toxicity profiles are usually described in textbooks as those of single, pure chemicals, and that toxicity of solvent mixtures is uncertain.

It is to be expected that the toxicity of solvent mixtures will vary depending on the toxicity of the individual solvent constituents. It

is, however, also true that toluene is the solvent present in the largest proportion in many cases,¹⁻³ suggesting that the major toxicity profile may be predominantly determined by toluene, with other solvents as minor toxicity modifiers.

The present study was initiated to investigate this possibility by examining dominant toxicity pictures among solvent workers exposed primarily to toluene, together with other common solvents such as ethyl acetate, methyl ethyl ketone (MEK), and isopropyl alcohol (IPA).

Materials and methods

WORKERS STUDIED

The study was conducted in the second half of working weeks (on Thursdays and Fridays). In total, 303 male solvent exposed workers and 136 male non-exposed controls were studied. The solvent exposed employees worked in printing, ink production, or surface treatment plants (where the surface of materials were treated with plastics dissolved in solvents). Control subjects were office workers and were recruited from clerical sections of the same factories. Efforts were made to collect controls with ages comparable with those of the exposed subjects; the control subjects were, however, on average older than the exposed subjects, although there was a considerable overlap in the age distributions (table 1).

COLLECTION OF SAMPLES

For the determination of the time weighted average (TWA) exposure concentration, each

Table 1 Demographic and exposure profile

Item	Exposed group	Control group
No	303	135
Age (y (SD))	32.9(11.4)	44.4 (11.2)
Exposure:		
Toluene	17.6 (3.40):479*	17.6 (3.40)/303†
MEK	5.3 (6.65):577	15.9 (3.34)/207
IPA	1.3 (4.67):274	6.7 (4.57)/108
Ethyl acetate	4.3 (5.57):741	8.6 (4.00)/229
Xylenes‡	0.7 (2.35): 38	6.3 (2.95)/ 32
Ethylbenzene	0.6 (2.27): 28	5.8 (2.72)/ 32
Sum§	0.31 (2.05): 8.4	0.33 (2.94)/303

*GM (GSD): the maximum (ppm), calculated with 303 determinations replacing 0 ppm with 0.5 ppm.

†GM (GSD) ppm/n, calculated with the cases excluding 0 ppm cases.

‡Sum of three xylene isomers.

§Summation of solvent concentrations from the additiveness formula,⁵ and based on the occupational exposure limits of 100 ppm for toluene, xylenes, and ethylbenzene, 200 ppm for MEK, and 400 ppm for IPA and ethyl acetate.²⁴

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solvent exposed worker was equipped with a diffusive sampler⁴ worn at chest level from the beginning of the shift (at around 0900) until the time of urine collection (at around 1600).

The shift ended at 1700. Near the end (at around 1600), they were invited to a solvent free examination room for sampling of venous blood and urine, and were requested to fill in subjective symptom questionnaires.

ANALYSIS OF SOLVENTS IN THE ENVIRONMENTAL AIR

After exposure, each carbon cloth in the sampler was extracted with carbon disulphide. The extract was analysed with an FID-gas chromatography apparatus as previously described.⁴ Because the solvent workers were exposed to more than one solvent in most cases, the sum (the summation index) was calculated from the additiveness formula⁵ for overall exposure evaluation.

URINALYSIS

Each worker was asked to pass urine between 1300 and 1400 and then urine discharged at between 1500 and 1600 was sampled. The samples were analysed for hippuric acid by high pressure liquid chromatography with an automated liquid sampler⁶ from which 2 μ l urine was introduced per injection after filtration through a membrane. The concentrations of the urinary metabolites were expressed as measured, or after correction for creatinine concentration⁷ or a specific gravity of urine of 1.016.⁸ Creatinine and specific gravity were measured by colorimetry and refractometry respectively.

CLINICAL EXAMINATIONS AND SUBJECTIVE SYMPTOM SURVEYS

The items studied in haematology were erythrocyte counts, leucocyte counts, thrombocyte counts, haemoglobin concentration, and packed cell volume, and those in serum biochemistry were aspartate aminotransferase (ASAT; EC2.6.1.1), alanine aminotransferase (ALAT; EC2.6.1.2), γ -glutamyl transpeptidase (γ -GTP; EC2.3.2.1), alkaline phosphatase (EC3.1.3.1), lactate dehydrogenase (LDH; EC1.1.1.27), leucine aminopeptidase (LAP; EC3.4.1.1), total cholesterol, high density lipoprotein cholesterol, triglyceride, blood urea nitrogen, uric acid, and creatinine. They were measured by conventional methods. For comparison between the exposed and control groups, the results were classified into three categories—normal, borderline, and abnormal cases—taking the normal/log-normal distribution of the values and clinical experiences into consideration as previously described.⁹ Activities of ASAT and ALAT were evaluated in combination in accordance with clinical practice.⁹

Subjective symptoms were surveyed by self administered questionnaires with 12 questions about symptoms during work and 57 questions about symptoms when not at work and in the past three months^{10,11}; the answers were confirmed in a medical interview. The

prevalence of the subjective symptoms was calculated⁹ as:

$$\frac{\text{number of affirmative answers by the group}}{(\text{number of the subjects in the group}) \times (\text{number of questions})} \times 100 (\%).$$

SELECTION OF THE EXPOSED SUBJECTS TO FORM SUBGROUPS BY COEXPOSURE TO ANOTHER SOLVENT

Among the 303 exposed workers, 207 subjects were exposed to MEK as well as toluene and other solvents, and 108 subjects were exposed to IPA as well as to toluene and others. To detect possible effects of MEK or IPA coexposure on toluene, MEK or IPA exposed subjects were matched by toluene exposure intensity with MEK or IPA non-exposed subjects. In practice, toluene exposure intensity was uneven between the MEK exposed and MEK non-exposed groups or between the IPA exposed and IPA non-exposed groups, and only 74 pairs (the +MEK group and the -MEK group) were available among MEK exposed and MEK non-exposed people, because only seven MEK exposed subjects were exposed to <4 ppm toluene by contrast with 29 MEK non-exposed subjects. For IPA exposure, only 95 pairs (the +IPA group and the -IPA group) were established primarily due to lack of high toluene exposure among the IPA exposed subjects.

STATISTICAL ANALYSIS

A log normal distribution was assumed for evaluation of the solvent vapour concentration.¹¹⁻¹⁴ In calculating the geometric mean (GM) and geometric SD (GSD), 0 ppm was replaced with 0.5 ppm for practical reasons. When the exposure-excretion correlation was calculated, the control group was employed to define the non-exposed level (the metabolite level without the exposure). In practice, the correlation was calculated utilising the combination of the control group with the exposed group. For statistical analysis, χ^2 test, Student's *t* test, regression analysis, and multivariate analysis were used.

Results

EXPOSURE TO ORGANIC SOLVENTS

Analysis of organic solvent vapours in ambient air collected by personal diffusive sampling (table 1) confirmed that the main solvent to which the workers were exposed was toluene. Ethyl acetate, MEK, and IPA were detected also in many but not all samples, depending on the workers studied. By contrast, xylenes and ethylbenzene were only found in a few samples.

The toluene concentration as a whole was rather low (GM 17.6 ppm), although some workers were exposed to up to 479 ppm. Of particular interest is the presence of MEK. Whereas this solvent was only detected in about two thirds of the total samples, the concentration was comparable to (GM 15.9 ppm) or even higher (577 ppm as the

maximum) than toluene concentration if it was present. When the sum of all the solvent concentrations was calculated from the additiveness formula (the summation index),⁵ the GM was about one third, whereas the maximum was in excess of eight times unity.

Statistical analysis (table 2) indicated that solvent concentrations were not independent of each other, and that there were significant

Table 2 Correlation among the solvent concentrations

	Ethyl- benzene	Xylenes	Ethyl acetate	IPA	MEK
Toluene	-0.076	-0.067	0.573**	0.871**	0.598**
MEK	-0.112	-0.107	0.057	0.647**	
IPA	-0.072	-0.071	0.443**		
Ethyl acetate	-0.067	-0.068			
Xylenes	0.971**				

** $p < 0.01$. Values are correlation coefficients.

Table 3 Distribution of normal, borderline, and abnormal cases in haematology and serum biochemistry

Item (unit)	Normal range	Control group	Exposed group
No of workers		135	303
Haematology:			
Haemoglobin (g/100 ml)	13.0-17.0	129/ 5/ 1	293/ 9/ 1
Leucocytes ($\times 10^3/\text{mm}^3$)	4.0- 8.0	123/12/ 0	287/16/ 0
Serum biochemistry:			
γ -GTP (IU/l)	6-45	107/13/15	262/23/18
ASAT and ALAT (IU/l)*	8-40, 5-35†	26/ 6/ 3	281/18/ 4

Values for groups are numbers of normal/borderline/abnormal cases. There was no significant difference in the distribution between control and exposed groups.

*ASAT and ALAT activities were evaluated in combination.⁹

†Normal ranges for both ASAT and ALAT are shown.

($p < 0.01$) correlations between toluene and MEK, toluene and IPA, toluene and ethyl acetate, IPA and MEK, IPA and ethyl acetate, and xylenes and ethylbenzene. Scattering of the concentrations around the regression lines were, however, wide, and the statistical significance of the correlations seemed to be due to large numbers of determinations. Figure 1 gives the scatter diagram of toluene *v* MEK and toluene *v* IPA. No significant ($p > 0.05$) correlation was detected between toluene and xylenes, toluene and ethylbenzene, or MEK and ethyl acetate. The lack of correlation in the first two cases was contrary to our expectation that xylenes and ethylbenzene might be present as toluene impurities.

CLINICAL SIGNS

Table 3 summarises results on haemoglobin concentrations, leucocyte counts, γ -GTP, ASAT and ALAT as examples of haematology and liver function. For haematology, a few examinees in the control group and the exposed group fell in the borderline category due to subclinical anaemia or subclinical leucocytosis, but the distribution pattern did not differ significantly ($p > 0.05$) between the two groups. γ -GTP concentrations were raised in about 15% of the examinees in both groups. There was no significant difference ($p > 0.05$) between the two groups. Activities of ASAT and ALAT were also increased in some 7% of the subjects studied, but with no significant ($p > 0.05$) difference in the distribution between the two groups.

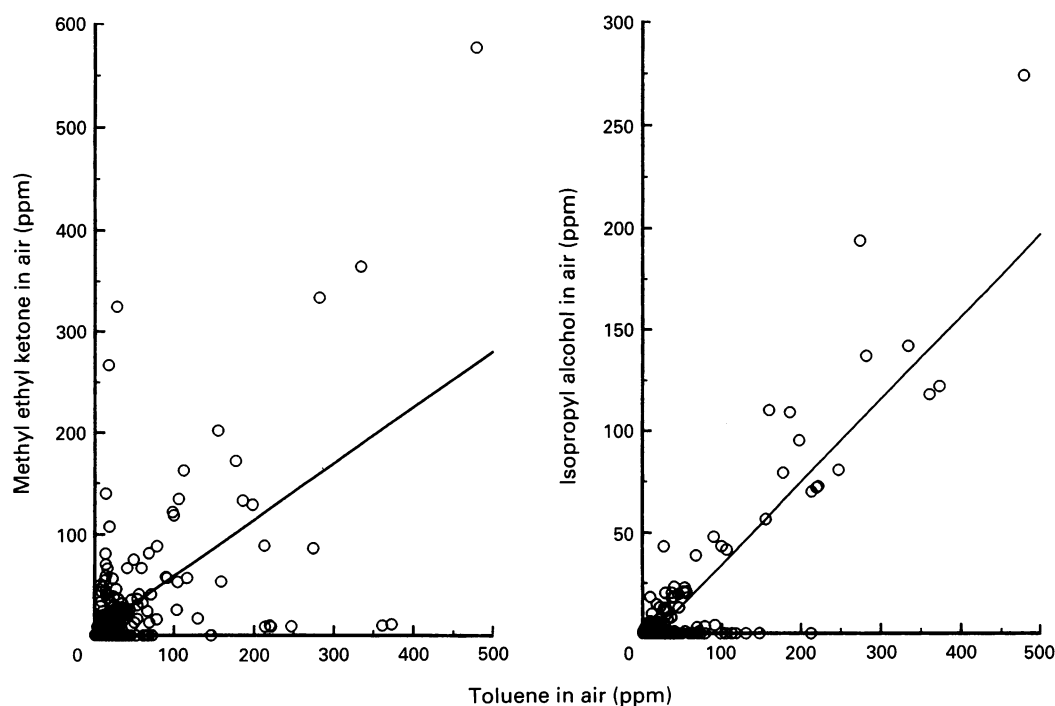


Figure 1 Scatter diagrams of the relation between toluene and methyl ethyl ketone (MEK), and toluene and isopropyl alcohol (IPA) in workroom air. (A) Toluene *v* MEK. (B) Toluene *v* IPA. The lines are calculated regression lines of $y = 3.34 + 0.554x$ ($r = 0.598$, $p < 0.01$, $n = 303$) and $z = -6.73 + 0.408x$ ($r = 0.871$, $p < 0.01$, $n = 303$), where x , y , and z are concentrations (ppm) of toluene, methyl ethyl ketone, and isopropyl alcohol, respectively.

Table 4 Prevalence of subjective symptoms

Subjective symptom	No of questions	Parameter	Controls	Exposed group†				
				Total	≤0.2	>0.2-0.5	>0.5-1.0	>1.0
No of subjects			135	303	78	135	56	34
During work	12	AA§	69	497	85	201	101	110
		Prevalence (%)¶	4.3	13.7**	9.1	12.4	15.0	27.0††
Not at work	57	AA	468	1320	341	473	270	236
		Prevalence (%)	5.9	7.4**	7.4	6.0	8.2	11.8††

**p < 0.01 v controls.

††p < 0.01 for difference among the four exposed subgroups.

‡Classification by summation of solvent concentrations as defined in the footnote to table 1.

§Numbers of affirmative answers.

¶For definition of prevalence of symptoms, see materials and methods.

INCREASE IN PREVALENCE OF SUBJECTIVE SYMPTOMS

Subjective symptoms were evaluated separately for those occurring during work and for those not at work. As well as the comparison between the exposed group and the non-exposed control group, the exposed subjects were divided into subgroups by use of the summation index as a total exposure indicator (table 4) for comparison among the subgroups of different exposure intensity. Whereas the subjective symptoms both during work and not at work were significantly

(p < 0.01) more prevalent in the exposed group than in the controls, the increase was larger for the symptoms during work (more than threefold) than for the symptoms not at work (well below twofold). The subgrouping of the exposed groups showed a significant (p < 0.01) dose associated increase in symptoms both during work and not at work. The prevalence of symptoms during work increased considerably when the summation index was in excess of 1, whereas the change in the prevalence was less remarkable when the symptoms not at work was studied.

Evaluation of each symptom during work (table 5) disclosed that the prevalence of seven symptoms out of the 12 was significantly (p < 0.01) greater in the exposed group than in the controls, and that dose dependency was confirmed in six symptoms among the seven. Graphical presentation of the dose response relation (fig 2) shows that the prevalence of two symptoms, irritation in eyes and nasal irritation, and possibly that of a drunken feeling increased when the exposure was above a certain level (0.5 to 1.0) whereas the prevalence of floating sensation was linearly increased as a function of exposure intensity.

By contrast, only two symptoms (dizziness and rough skin) showed significant (p < 0.01) dose dependency among the 57 symptoms when not at work. The difference in the prevalence between the exposed group and the control group was, however, significant (p < 0.01) for nine symptoms (table 5).

Table 5 Symptoms during work with significant difference in prevalence

Subjective symptoms‡	Prevalence§		Influential solvents¶	
	Controls	Exposed	The most	The 2nd most
During work:				
1 Irritation in eyes	8.1	19.8***††	Toluene	
3 Nasal irritation	1.5	37.3***††	Ethyl acetate	MEK
4 Unusual smell	1.5	26.1***††	Toluene	
5 Sore throat	4.4	15.5**	(None)	
6 Unusual taste	0.7	5.3***††	Toluene	
9 Floating sensation	2.2	14.5***††	Toluene	
10 Drunken feeling	1.5	14.5***††	Toluene	
When not at work:				
103 Dizziness	1.5	5.6***††	Toluene	IPA
110 Lightheadedness	0.0	7.3**		
111 Muddleheadedness	3.0	11.9**		
118 Fainting after sudden standing up	5.2	15.2**		
122 General dullness	8.1	25.7**		
123 Dullness in extremities	0.7	8.3**		
130 Body weight loss	4.4	12.2**		
141 Reduced sense of smell	2.2	11.2**		
151 Rough skin	8.1	21.8***††	IPA	

**p < 0.01 v controls; †† p < 0.01 for difference among subgroups when the exposed group was classified into those exposed at ≤0.2, >0.2-0.5, >0.5-1.0, and >1.0 (unity calculated by the additiveness formula).

Symptoms listed are those with significant differences (p < 0.01) in the prevalence between the control and exposed groups.

‡Symptoms are coded after Uchida *et al.*¹¹

§Values are prevalence in % for the symptom prevalence as defined in materials and methods.

¶Solvents were identified by multivariate analysis, taking six solvent concentrations as independent variables. Calculations were made only in cases shown by two daggers.

Table 6 Solvent exposure of subgroups as classified by exposure to MEK and IPA

Exposure to solvents	Classification by			
	MEK		IPA	
	+MEK	-MEK	+IPA	-IPA
No	74	74	95	95
Toluene	15.8 (2.68)	15.2 (2.76)	20.3 (2.51)	20.3 (2.52)
MEK	12.1 (3.48)	—	10.0 (5.09)**	4.6 (5.92)
IPA	1.3 (3.35)**	0.7 (2.51)	4.6 (3.18)	—
Ethyl acetate	4.5 (3.75)**	1.5 (4.96)	8.3 (4.48)**	2.9 (4.41)
Xylenes	0.6 (1.61)**	1.3 (4.17)	0.5 (1.53)**	0.8 (3.11)
Ethylbenzene	0.6 (1.66)*	0.8 (2.84)	0.6 (1.66)**	0.8 (2.84)

*p < 0.05; **p < 0.01 v the paired value.

Values are GM (GSD) ppm.

Table 7 Symptoms by subgroups as classified by exposure to MEK and IPA

Subjective symptom*	Classification by			
	MEK		IPA	
	+MEK	-MEK	+IPA	-IPA
During work:				
1 Irritation in eyes	23.0	16.2	16.8	22.1
3 Nasal irritation	39.1	23.0	45.3	35.8
4 Unusual smell	28.3	20.3	33.7	26.3
5 Sore throat	16.2	9.5	15.8	17.9
6 Unusual taste	12.2	2.7	5.3	7.4
9 Floating sensation	17.5	9.5	14.7	14.7
10 Drunken feeling	12.2	8.1	12.6	14.7
Not at work:				
103 Dizziness	2.7	5.4	2.1	9.5
151 Rough skin	17.6	14.9	15.8	14.7

Values are prevalence (%).

*Symptoms are coded after Uchida *et al.*¹¹

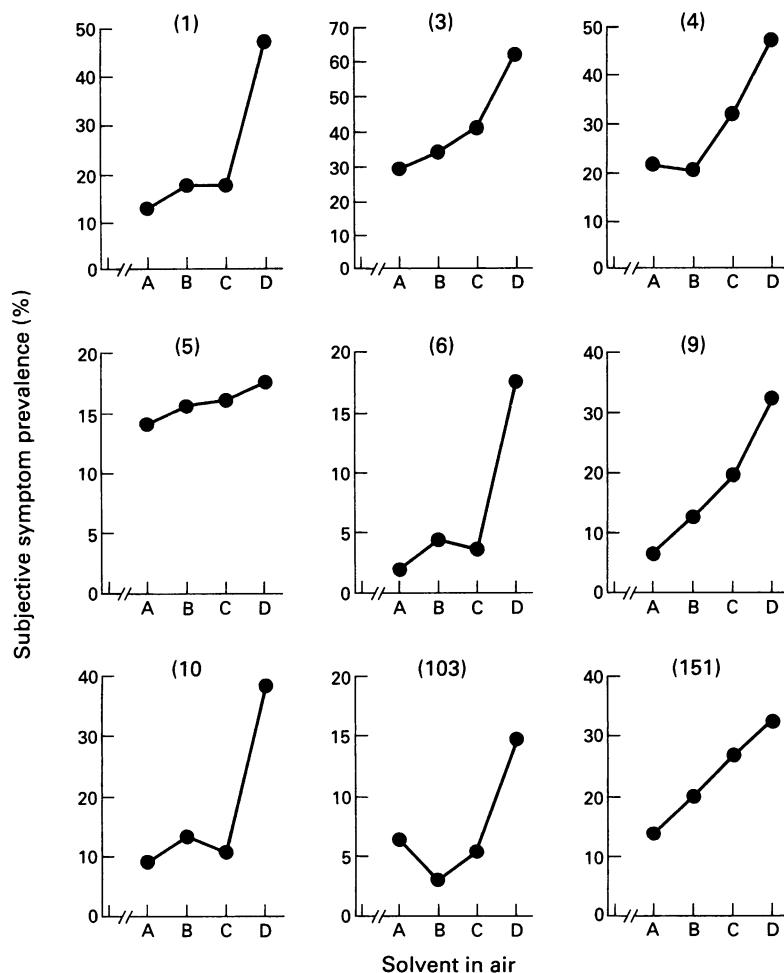


Figure 2 Symptoms during work and when not at work, by intensity of exposure. Symptoms with code numbers of 1 to 10 are symptoms during work, and the code numbers 103 and 151 are symptoms when not at work. Exposure intensity is expressed in terms of unity from the additiveness formula²—that is, A \leq 0.2, B > 0.2–0.5, C > 0.5–1.0, and D > 1.0. The numbers of workers in the subgroups A, B, C, and D are 78, 135, 56, and 34 respectively. For definition of prevalence, see materials and methods.

Trials were made to identify the most influential solvent in inducing each symptom. For this purpose, multivariate analysis was performed taking each solvent concentration as independent variable (although they were in fact not independent in many cases as shown in table 2). Toluene was the most

powerful determinant in almost all the symptoms (table 5).

SEARCH TO IDENTIFY ANY SYMPTOMS

ATTRIBUTABLE TO EXPOSURE TO MEK OR IPA

Among the solvents copresent with toluene, MEK and IPA were the most common when the least toxic ethyl acetate¹⁵ was excluded (table 1). Accordingly, trials were made to detect possible symptoms attributable to the toxicities of these solvents, by comparing the symptom prevalence among those exposed to MEK or IPA with those not exposed to MEK or IPA. In practice, 74 pairs of those exposed to MEK (the +MEK group) and those not exposed to MEK (the -MEK group), as well as 95 pairs of those exposed to IPA (the +IPA group) and those not exposed to IPA (the -IPA group) were established (for details of procedures, see materials and methods). Table 6 summarises the exposure of these subgroups to the solvents. It should be noted that the toluene exposure was similar between the +MEK group (GM 15.8 ppm and the -MEK group (15.2 ppm), and also between the +IPA group (20.3 ppm) and the -IPA group (20.3 ppm).

Comparison of subjective symptom prevalence between the +MEK group and the -MEK groups (table 7) showed that none of the symptom prevalences differed significantly ($p > 0.05$), although the +MEK group tended to have more symptoms than the -MEK group. A similar analysis of the +IPA group in comparison with the -IPA group did not show any significant ($p > 0.05$) increase in symptoms in association with IPA exposure (table 7).

REGRESSION ANALYSIS OF THE EXPOSURE-EXCRETION RELATION IN TOLUENE METABOLISM

Toluene was present in all of the 303 solvent exposed samples studied. Accordingly, the relation of intensity of exposure to toluene with hippuric acid (a major toluene metabolite) in the end of shift urine samples was examined, ignoring the coexposures to other solvents. Calculation showed (table 8) that hippuric acid concentrations correlated significantly ($p < 0.01$) with TWA exposure concentrations of toluene, regardless of whether the values were corrected for urinary creatinine concentration or specific gravity.

The relation between toluene in air and hippuric acid in urine was examined in the +MEK group in comparison with the -MEK group. The comparison of the slopes between the two groups showed (table 9) that there was no significant ($p > 0.05$) difference in the slopes when urinary hippuric acid concentrations were either uncorrected or corrected for specific gravity, although the difference was significant ($p < 0.05$) when the concentrations were corrected for creatinine concentration. An analogous calculation showed no significant differences between the +IPA group and the -IPA group (table 9).

Table 8 Correlation of urinary hippuric acid excretion with environmental toluene exposure: comparison between single and mixture exposure

Case	Regression parameter		
	α	β	r
The present study with mixed exposure:†			
No correction (observed value) (mg/l)	747 (653–1417)	14.9 (13.3–16.5)	0.655
Corrected for:			
Creatinine (mg/g creatinine)	536 (486–583)	13.9 (13.1–14.7)	0.840
Specific gravity (1.016) (mg/l)	495 (448–542)	8.0 (7.2–8.8)	0.686
Exposure to toluene only:‡			
No correction (observed value) (mg/l)	271 (-2–544)	31.1 (24.9–37.3)	0.803
Corrected for:			
Creatinine (mg/g creatinine)	361 (116–607)	15.8 (10.2–21.4)	0.606
Specific gravity (1.016) (mg/l)	273 (114–433)	16.2 (12.6–19.8)	0.768

* α and β are parameters of a calculated regression line so that $y = \alpha + \beta x$, where x is toluene in air (ppm), and y is hippuric acid in an end of shift urine sample as shown in the table. All correlation coefficients (r) are statistically significant ($p < 0.01$). Values in parentheses indicate the 95% CI.

†With a total of 303 exposed workers and 135 non-exposed workers.

‡Values for male workers are cited from Hasegawa *et al.*²⁶

Table 9 Absence of effect of coexposure to MEK or IPA on the correlation of urinary hippuric acid excretion with environmental toluene exposure

Case	Regression parameter					
	α		β		r	
	With	Without	With	Without	With	Without
Correction for urine density						
Effect of coexposure to methyl ethyl ketone:						
No correction (observed value) (mg/l)	505	463	26.1	22.6	0.549	0.609
Corrected for:						
Creatinine (mg/g creatinine)	343	348	23.7*	19.5	0.783	0.736
Specific gravity (1.016) (mg/l)	327	320	16.2	14.4	0.640	0.628
Effect of coexposure to isopropyl alcohol:						
No correction (observed value) (mg/l)	571	575	27.8	15.7	0.618	0.520
Corrected for:						
Creatinine (mg/g creatinine)	381	382	23.2	17.6	0.841	0.769
Specific gravity (1.016) (mg/l)	367	367	16.3	10.7	0.703	0.611

* $p < 0.05$ v the counterpart value. The regression lines (as defined in footnote to table 8) were calculated with 209 cases (the +MEK group and the control group, or the -MEK group and the control group) for MEK coexposure, and with 230 cases (the +IPA group and the control group, or the -IPA group and the control group) for IPA coexposure. With = With coexposure to MEK or IPA; without = Without coexposure.

Discussion

Several articles have been published recently on the health effects of exposure to solvent mixtures. It is conceivable that the general picture of the effects will vary depending on the composition of the solvent mixture, and the intensity of the effects should be a function of the extent of exposure.

The published reports suggest two organs as the targets of toxicity—namely, the nervous system and the liver. For example, Houck *et al*¹⁶ described two cases of memory and concentration impairment after exposure to solvent mixtures during polychlorinated biphenyl clearing work. There were two types of solvent mixtures used; one consisted of alkylated benzenes and monoethanolamine, and the other was a mixture of methylchloroform and tetrachloroethylene. Unfortunately the solvent preparations the workers used were not specified. The workers were probably also exposed to polychlorinated biphenyls, although it is not likely that the symptoms were attributable to these. Lindelof *et al*¹⁷ found a significant increase in the prevalence of sleeping difficulties among solvent exposed workers, but no detailed description of solvent composition or intensity of exposure was given. Murata *et al*¹⁸ by contrast, noted dysfunction of the autonomic and peripheral nervous system in a group of 11 solvent exposed workers. Most of them were exposed to toluene together with other solvents. Interestingly, only two of them were coexposed to *n*-hexane, a well established neurotoxin.¹⁹ The intensity of exposure was, however, not described.

Lundberg and Hakanenonson²⁰ examined paint industry workers with heavy exposure to solvent mixtures (toluene and xylenes being the main constituents but alcohols and others were also present; the sum after the additiveness formula⁵ was three or more). They found no significant increase in ASAT, ALAT, or γ -GTP in serum of the exposed workers. Similarly, Chen and others²¹ detected no changes in ASAT, ALAT, and other enzymes in the serum of the solvent exposed workers,

but γ -GTP increased in parallel with the summation index. The solvent exposure was heavy; the highest concentrations recorded were 948 ppm for toluene, 511 ppm for xylenes, and 138 ppm for benzene, and the summation index was as high as 9.8.

The present study was conducted on factory workers exposed to MEK, IPA, and ethyl acetate as well as toluene and its homologues of xylenes and ethylbenzene (table 1). The clinical signs in haematology and liver function were essentially negative (table 3) as is the case after exposure to toluene alone.²² Subjective symptoms during work included a floating sensation and a drunken feeling, which are related to the suppressive effects on the central nervous system, and the picture was again similar to that recorded for toluene exposure.²²

Perusal of symptom profiles suggest that many workers complained of local irritation, typically to the eyes and the nose (table 5). Because MEK, one of the coexposed solvents, is a known local irritant,²³ trials were done to examine if the irritation in the eyes and the nose (seen among the exposed workers) were related to exposure to MEK. Multivariate analysis showed that toluene was the most powerful determinant of eye irritation symptoms whereas MEK was the second most powerful one for nasal irritation (table 5). Further analysis controlling toluene concentration (table 6) showed that MEK was not an irritant under the exposure conditions studied. This is most probably due to the fact that MEK concentration was low in this study (GM 16 ppm; table 1) compared with the current odour and irritation based occupational exposure limit of 200 ppm.^{5,23,24} In this connection, it is of interest to note the conclusion of Dick and others²⁵ that no additivity was evident between toluene and MEK in psychomotor tests after experimental exposure of human volunteers either to toluene alone (100 ppm), to MEK alone (200 ppm), or to the combination of 50 ppm toluene and 100 ppm MEK.

The present study showed that the urinary hippuric acid concentrations are linearly related to toluene exposure concentration even under the conditions of coexposures to MEK, IPA, and other solvents (table 8). Statistical evaluation was not possible on whether or not the slopes of the regression lines after mixture exposure were less than the slopes after exposure to toluene alone.

Comparison with the values reported in the literature²⁶ however, did not suggest this possibility, because there is significant overlapping in the 95% confidence intervals (95% CIs) of the regression lines (table 8). Furthermore, the comparison of slopes between those coexposed to either MEK or IPA and those not coexposed show that there was no reduction in the slopes due to the coexposure (table 9). It has previously been shown that metabolism of toluene and xylenes is independent¹⁴ when exposure intensity is below the current occupational exposure limit of 100 ppm for each solvent,^{23,24} although both solvents share

methyl moiety oxidation followed by glycine conjugation in their metabolism. By contrast, both MEK and IPA have chemical structures different from the two methylated aromatics and both may undergo glucuronidation either directly or after reduction at the oxygen.²⁷ Such differences in chemical structure and metabolism coupled with low exposure are in line with the lack of metabolic interaction of MEK and IPA with toluene.

In evaluating the lack of metabolic interaction in the present study, low concentrations of MEK (GM 16 ppm, table 1) and IPA (GM 7 ppm) should be taken into account. Tardif *et al*²⁸ did not find metabolic interaction between 50 ppm toluene and 40 ppm xylene when human volunteers were exposed to the vapour either separately or in combination, although they detected interaction between toluene at 95 ppm and xylene at 80 ppm. In a factory survey, Kawai *et al*²⁹ found that individual solvent metabolism was not influenced by the presence of other solvents when workers were exposed to toluene, styrene, and methanol in combination at low concentrations (GMs 7.3, 4.7, and 15.9 ppm respectively). The present finding is consistent with those of Tardif *et al*,²⁸ Kawai *et al*,²⁹ and Huang *et al*¹⁴ in supporting the hypothesis that there will be no metabolic interaction when the exposure to a solvent mixture is not great. Bearing in mind that the exposure intensity in the present study is well below the current occupational exposure limit even when additiveness is assumed, it is desirable to investigate possible modification of toxicity and metabolism at exposures around the occupational exposure limit.

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