ORIGINAL ARTICLE

Are women more sensitive than men to 2-propanol and *m*-xylene vapours?

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Aims: To evaluate possible differences between men and women in acute health effects after controlled short term chamber exposure to vapours of two common organic solvents.
Methods: Fifty six healthy volunteers (28 per sex) were exposed to 150 ppm 2-propanol, 50 ppm *m*-xylene, and clean air for two hours at rest. The subjects rated symptoms on a visual analogue scale before, during, and after the exposure. Blinking frequency was measured continuously during exposure. Pulmonary function, nasal swelling, inflammatory markers (lysozyme, eosinophilic cationic potein, myeloperoxidase, albumin) in nasal lavage and colour vision (Lanthony D-15 desaturated panel) were measured before and at 0 and 3 hours after the exposure.
Results: There were no significant sex differences in response to solvent exposure with respect to blinking frequency, lung diffusing capacity, nasal area and volume, inflammatory markers in nasal lavage, and colour vision. Increased symptoms were rated by both sexes for nearly all 10 questions during exposure to 2-propanol or *m*-xylene, most increases being significant at one time point at least. The rating of "discomfort in the throat or airways" increased more in women during exposure to 2-propanol or *m*-xylene. During exposure to 2-propanol the rating of "fatigue" was more increased in men after one hour, but more increased in women after two hours of exposure. With regard to pulmonary function, women had small but significant decreases in FVC, FEV_1/FVC, and FEF_{75} three hours after exposure to *m*-xylene, but only the decrease in FVC was significantly different from that in men.
Conclusion: Our results suggest that women are slightly more sensitive than men to the acute irritative effects of 2-propanol and *m*-xylene vapours.

In recent years, women in Sweden and other Western European countries have started to work in industrial settings to a larger extent; occupational exposure of women to organic solvents may thus be becoming more common. It has been estimated that about 9% of the Swedish workforce are exposed to solvents at least one fourth of the working time, and one third of these are females.[1] Assessment of health effects of solvents has, however, almost exclusively been based on studies in males, and knowledge of the relation between women's exposure to solvents and their health effects is minimal.[2] Results from studies in men are therefore generalised to women. This may lead to misjudgement of the health risks for women.

The objective of this study was to evaluate possible differences in acute health effects in men and women after controlled short term chamber exposure to vapours of two common organic solvents, 2-propanol and *m*-xylene. The two solvents were selected according to three criteria: (1) they are considerably different in lipophilicity and hydrophilicity; (2) they are commonly used; and (3) they are without serious health risks at relevant exposure levels.

2-Propanol is a hydrophilic solvent that is widely used in industry and households, for example, as a disinfectant at home, in hospitals, and in industry, as a solvent in the production of hair and skin products, as an antifreeze agent in fuel systems, in windshield washers, in lens cleaners, and in racing motor fuels. The critical effect of 2-propanol is irritation of the respiratory system, eye, and mucous membranes. Higher concentrations cause central nervous system effects such as dizziness, nausea, hypotension, and hypothermia.[3] Irritation in the nose and throat has been reported at exposure levels around 400 ppm.[4] The Swedish occupational exposure limit (OEL, eight hour time weighted average) for 2-propanol is 150 ppm,[5] whereas the threshold limit value (TLV) given by the American Conference for Governmental Industrial Hygienists (ACGIH) is 400 ppm.[6]

Xylene is a lipophilic solvent used in paints and in the production of phthalic anhydride, plasticisers, and polyesters. The critical effects of xylene are depression of the central nervous system (for example, headache, nausea, fatigue), and irritation of the upper respiratory system and the eyes.[7] The no observed adverse effect level for acute central nervous system (CNS) effects in humans is about 70 ppm for a four hour exposure.[7] The Swedish OEL and ACGIH TLV for xylene are 50 ppm and 100 ppm respectively.[5 6]

Tests for effects in this kind of study have to be simple and acceptable to the subjects and thought to be sensitive to solvent effects. We thus used questionnaires with visual analogue scales to allow graded ratings of irritation and CNS symptoms. In addition, irritation in the airways was assessed by pulmonary function and transfer tests. Mucous membrane irritation in the nose was monitored by acoustic rhinometry, a well documented technique[8] that has sensivity to solvents.[9] Blinking frequency was measured as an indicator of eye irritation. Colour vision was measured as a sensitive and quantitative indicator of early neurotoxic effects. Increased colour vision deficit has been shown in solvent exposed workers[10 11] and in an experimental study by Baelum and colleagues.[12] In addition, proteins in nasal lavage were

Abbreviations: ANOVA, analysis of variance; CCI, Colour Confusion Index; CNS, central nervous system; DL_{CO}, diffusing capacity for carbon monoxide in the lung; ECP, eosinophilic cationic protein; EMG, electromyography; FEF, forced expiratory flow; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; MPO, myeloperoxidase; OEL, occupational exposure limit; PEF, peak expiratory flow; TLV, threshold limit value; VAS, visual analogue scale; VC, vital capacity

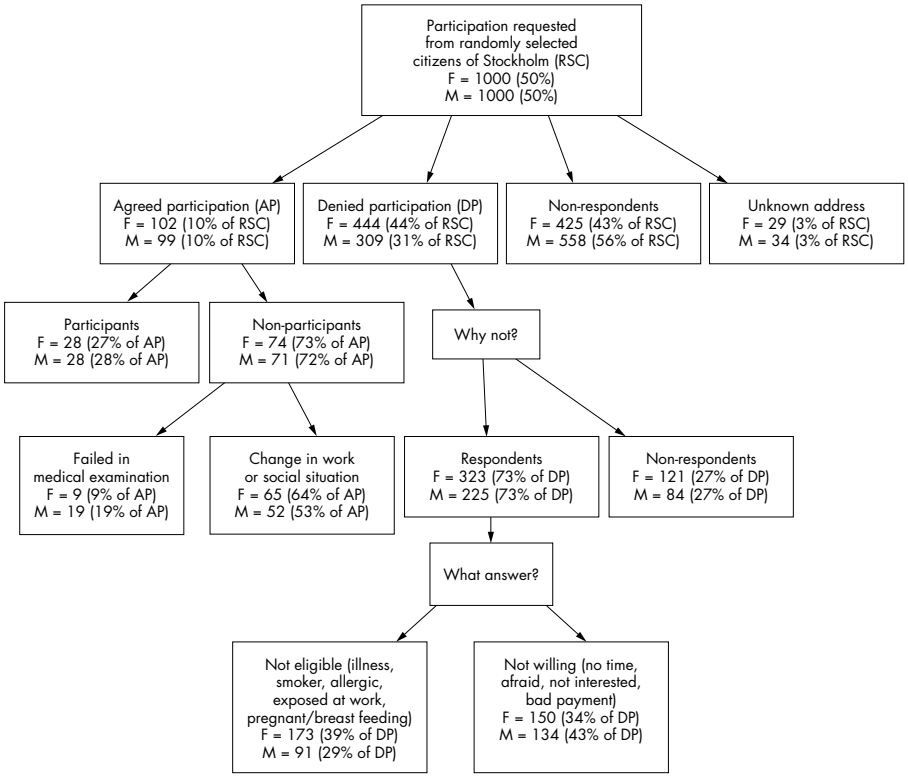

Figure 1 Selection procedure for the study group.

analysed to study inflammatory response. The markers analysed were myeloperoxidase (MPO), a specific biomarker of the activity of neutrophil granulocytes[13]; eosinophilic cationic protein (ECP), a marker of the activity of eosinophil granulocytes; lysozyme, secreted from submucosal glands, macrophages, and neutrophil granulocytes[14]; and albumin, which indicates plasma leakage.

METHODS

Subjects

Fifty six white volunteers (28 men and 28 women) with a mean age of 34 years for both sexes (range 20–49 years), participated in the chamber exposure study. The volunteers were recruited from a randomly selected subgroup between 20 and 50 years of age from the Stockholm population registry (SPAR, Sweden). Figure 1 shows the recruitment procedure. Smokers and subjects with occupations or hobbies associated with exposure to organic solvents, or with a history of allergy or other chronic diseases were excluded. The subjects had to be healthy as judged by medical examination, and clinical blood chemistry. A general diagnostic test for atopy containing common environmental airway allergens (Phadiatop, Pharmacia & Upjohn, Sweden) indicated that none of the subjects were atopic. The blood diagnostic tests were performed by Nova Medical, Stockholm, Sweden. Females underwent a pregnancy test (urine human chorionic gonatropin, Boehringer Mannheim, Italy) immediately before each exposure. The volunteers were informed orally and in writing about the design of the study, possible hazards, and their freedom to discontinue whenever they wanted. Each participant signed an informed consent form. The study was approved by the regional ethical committee at the Karolinska Institute.

Experimental design

One to four subjects at a time were exposed on three different occasions, to 2-propanol at 150 ppm (350 mg/m^3), *m*-xylene at 50 ppm (200 mg/m^3), or clean air (control exposure). Each exposure lasted two hours and was conducted during resting conditions with the subjects seated. Subjects were allowed normal social interactions during the session. They were exposed in different exposure orders, and exposure sessions were separated by at least two weeks.

The experiment was initially designed to be well balanced—that is, with two women and two men exposed on each occasion and with all six exposure orders represented. Along the course of the study, however, several volunteers postponed participation for different reasons. This resulted in a varying number of women and men present at each exposure occasion and an imbalance in the exposure order. Thus, 28 subjects started with the control condition, 20 with 2-propanol, and eight with *m*-xylene exposure. We therefore tested for potential bias caused by this imbalance. The ANOVA analysis (see the section on statistical analyses) showed no significant

Table 1 Exposure conditions in chamber air

	2-propanol		*m*-xylene		Control	
	Women (n=28)	Men (n=28)	Women (n=28)	Men (n=28)	Women (n=28)	Men (n=28)
Temperature, °C	23.8 (0.8)	23.7 (0.6)	24.0 (0.9)	24.0 (0.6)	23.9 (0.9)	23.6 (0.9)
Humidity, % RH	26.7 (1.4)	27.2 (3.0)	29.0 (6.2)	29.0 (6.1)	28.0 (63.3)	27.6 (3.9)
Target concentration, mg/m^3	350	350	200	200	–	–
Measured concentration, mg/m^3	345.5 (16.1)	347.6 (16.6)	198.1 (6.5)	196.4 (7.2)	–	–

Values expressed as mean (SD).

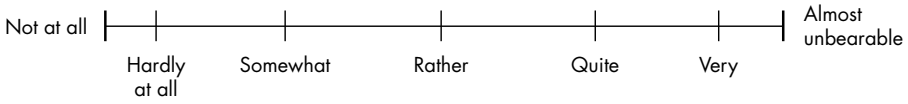

Figure 2 The visual analogue scale (0–100 mm) used for symptom ratings in the questionnaire. In the study, all verbal gradings and questions were written in Swedish.

influence of the order of exposure on the effect variables. Furthermore, no systematic difference was seen between the exposure conditions with respect to the number of women and men present per exposure occasion.

Exposure chamber

The exposures were carried out in a 20 m^3 dynamic exposure chamber with 18–20 air changes per hour. Temperature, relative humidity, carbon dioxide level, and outlet flow rates of chamber air were continuously monitored via an analogue digital converter (Squirrel Meter Logger 1200 Series, Grant Sweden). The temperature and the humidity in the chamber were set to 24°C and 30%, respectively. To avoid leakage of solvent vapour into the surrounding laboratory the outlet airflow rate was set slightly higher than the inlet rate. Solvent vapour was generated by injecting liquid solvent into inlet air by means of a high pressure piston pump (Gilson 302, France). The inlet air was dispersed throughout the entire chamber ceiling, and two fans in the chamber further secured an even distribution of solvent vapours in the chamber.

Air was sampled from the upper central part of the exposure chamber to monitor the concentration of solvent during exposures. The air samples were transferred through a Teflon coated tube to a gas chromatograph by means of a pump (DDA-P101-BN, Gast, Benton Harbor, USA). The gas chromatograph (Auto system, Perkin Elmer) was equipped with a wide bore capillary column (CP-sil 52, 25 m, 0.53 ID, 2 µm, Chrompack) and a flame ionisation detector. Nitrogen was used as a carrier gas at a flow rate of 2.5 ml/min. The temperature of the oven was 225°C and of the detector 250°C.

There were no appreciable differences between the three exposure conditions with regard to mean temperature and humidity. Moreover, there was no difference between sexes with regard to mean temperature, humidity, and solvent exposure levels (table 1).

Symptom ratings

The subjects rated the level of perceived discomfort in a questionnaire with 10 questions, immediately before, during (3, 60, and 118 minutes from start of exposure), and post exposure (140 and 350 minutes from onset of exposure). The 10 questions in Swedish were: (1) "discomfort in the eyes: burning, irritated, or running eyes"; (2) "discomfort in the nose: burning, irritated, or runny nose"; (3) "discomfort in the throat or airways"; (4) "breathing difficulty"; (5) "solvent smell"; (6) "headache"; (7) "fatigue"; (8) "nausea"; (9) "dizziness"; and (10) "feeling of intoxication". The ratings were performed using a 0–100 mm visual analogue scale (VAS, fig 2) graded from "not at all" (corresponding to 0 mm) through "hardly at all" (6 mm), "somewhat" (26 mm), "rather" (48 mm), "quite" (71 mm), "very" (90 mm) to "almost unbearable" (100 mm). The present questionnaire was elaborated for vapour exposure and has been used in several similar inhalation studies performed in our laboratory.[15–17]

Pulmonary function

Pulmonary function measurements were performed prior to, immediately after, and at three hours post-exposure. Measurements included vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), peak expiratory flow (PEF), and forced expiratory flow in 25%, 50%, and 75% of FVC (FEF_{25}, FEF_{50}, FEF_{75}). Secondary parameters calculated from the spirogram were FEV_1 /FVC and FEV_1 /VC. The measurements were performed according to a standard procedure,[18] using a spirometer (Vitalograf 21210; Buckingham, England,) along with computer software (Spirotrac 3, version 2.0).

Measurements of diffusing capacity for carbon monoxide in the lung (DL_{CO}) were performed according to the single breath holding method.[18 19] The subject inhaled a maximal breath of a test gas containing carbon monoxide (0.3%) and helium (14%), held it in the lungs for eight seconds, and exhaled. A sample of the exhaled alveolar gas was taken for analysis. DL_{CO} was measured prior to exposure and at 20 minutes post-exposure.

Acoustic rhinometry

Nasal swelling was assessed by acoustic rhinometry before, immediately after, and at three hours after exposure. The instrument and software (Nasal Area Distance Acquisition

Table 2 Colour vision and symptom ratings in non-exposed women and men

Question	Women (n=28)	Men (n=28)	p value*
Color confusion index†	1.14	1.25	0.04
Symptom ratings‡			
1 Discomfort in the eyes	3.4	1.3	–
2 Discomfort in the nose	4.6	2.6	–
3 Discomfort in throat or airways	7.1	2.5	0.06
4 Breathing difficulty	3.1	1.0	–
Average of symptoms 1–4	4.5	1.9	0.07
5 Solvent smell	1.3	0.6	–
6 Headache	2.3	1.1	–
7 Fatigue	11.0	7.3	–
8 Nausea	1.1	0.8	–
9 Dizziness	0.7	0.9	–
10 Feeling of intoxication	0.5	0.7	–
Average of symptoms 6–10	3.3	2.4	–

*p value in Mann-Whitney U test, p value only given when <0.1.
†One male with congenital colour blindness excluded.
‡Ratings in mm on a 0–100 mm visual analogue scale, average of three ratings per subject prior to exposure.

Table 3 Average symptom ratings during exposure to 150 ppm 2-propanol (2P)*

	60 min from start of exposure							118 min from start of exposure						
	Women (n=28)			Men (n=28)				Women (n=28)			Men (n=28)			
Question	Clean air	2P	p value, 2P *v* clean air†	Clean air	2P	p value, 2P *v* clean air†	p value, women *v* men‡	Clean air	2P	p value, 2P *v* clean air†	Clean air	2P	p value, 2P *v* clean air†	p value, women *v* men‡
Discomfort in the eyes	3.6	10.2	0.01	3	5.6	–	–	5.8	8.1	–	2.8	6.1	0.01	–
Discomfort in the nose	3.6	11.5	0.001	2.8	7.5	0.03	–	3.7	10.5	0.004	2.5	7.1	0.0006	–
Discomfort in the throat or airways	5.9	13.9	0.004	3.4	4.5	–	0.04	7.6	10	–	2.7	5.9	0.03	–
Breathing difficulty	3.6	7.8	0.004	1.8	2.7	–	0.08	2.3	6.1	0.09	1.2	1.8	–	–
Solvent smell	4.5	58.8	<0.0001	1.9	48.8	<0.0001	–	2.5	50.8	<0.0001	1.2	43.3	<0.0001	0.08
Headache	4.3	7.6	0.02	2.2	5.9	0.04	–	4.9	7.9	0.06	2.6	6	0.04	–
Fatigue	20.4	22.7	–	8	12.7	–	0.02	19.8	24.1	–	12.3	14.2	–	0.04
Nausea	2.2	2.2	–	1.4	3.7	–	–	1.9	2.7	0.08	0.9	2	0.03	–
Dizziness	2.8	3.4	–	2	4.6	–	–	2.4	4.2	0.05	0.8	3.3	0.01	–
Feeling of intoxication	1.3	4.4	0.06	1.4	3.9	–	–	1.5	3.7	0.03	1.2	1.6	–	–

*Ratings in mm on a 0–100 mm visual analogue scale (VAS).
†Wilcoxon signed rank test, p value only given when <0.1.
‡Mann-Whitney U test, p value only given when <0.1.

Program, version 1.3) used have been described previously.[20] The nasal volume and the minimal nasal cross sectional area were determined as an average of three measurements in each nostril. The recordings were performed after at least 30 minutes acclimatisation to room temperature. In the subsequent analyses the sum of the values from both nostrils were used.

Inflammatory markers

Inflammatory markers were measured in nasal lavages before and at three hours post- exposure. The lavages were performed after the acoustic rhinometry measurements as previously described by Nihlen and colleagues.[21] The analyses included MPO, ECP, lysozyme, and albumin, and were carried out at the Department of Clinical Chemistry, University Hospital, Uppsala, Sweden.

Blinking frequency

Eye blinking was monitored throughout the two hour exposure by electromyography (EMG). Three EMG electrodes were affixed around the left eye. The EMG signal was amplified and transferred via telemetry to a personal computer.[22] A software program in C++ was used for identification of the characteristic EMG signal pattern. Identification of eye blinks was performed by comparison against nine conditions related to the size, shape, and appearance of the pattern. Results were presented as blinks per minute in 20 minute intervals during the exposure.

Colour vision

Colour vision was assessed with the Lanthony D-15 desaturated panel colour arrangement test.[23] The test was performed before, immediately after, and at three hours post-exposure. The subjects used both eyes when performing the test; those who normally used spectacles wore them during the test. One of the subjects had congenital colour blindness. Quantitative evaluation was made by calculating the Colour Confusion Index (CCI).[24]

Statistical analyses

As the first step in the analyses, the distribution of each variable was tested using the Shapiro-Wilk test for normality (JMP version 3.02, SAS Institute Inc.). This test showed that the VAS ratings and CCI values (before conversion to percentage change) were not normally distributed. Therefore, the Wilcoxon signed rank test and the Mann-Whitney U test in the StatView (version 5, SAS Institute Inc.) software package were used to test for differences in symptom ratings between exposure conditions and sexes, respectively (tables 2, 3, and

Table 4 Average symptom ratings during exposure to 50 ppm *m*-xylene (mX)*

	60 min from start of exposure							118 min from start of exposure						
	Women (n=28)			Men (n=28)				Women (n=28)			Men (n=28)			
Question	Clean air	mX	p value, mX *v* clean air†	Clean air	mX	p value, mX *v* clean air†	p value, women *v* men‡	Clean air	mX	p value, mX *v* clean air†	Clean air	mX	p value, mX *v* clean air†	p value, women *v* men‡
Discomfort in the eyes	3.6	8.8	0.04	3	7.4	0.01	–	5.8	10.4	0.04	2.8	8.6	0.004	–
Discomfort in the nose	3.6	14.2	0.0003	2.8	8.3	0.003	–	3.7	15.6	0.004	2.5	11.5	0.001	–
Discomfort in the throat or airways	5.9	14.5	0.003	3.4	6.9	–	0.02	7.6	14.6	0.09	2.7	5.1	0.08	0.09
Breathing difficulty	3.6	6.8	0.009	1.8	7.4	0.008	–	2.3	6.8	–	1.2	4.2	0.003	–
Solvent smell	4.5	49.6	<0.0001	1.9	41.4	<0.0001	–	2.5	44.8	<0.0001	1.2	35.1	<0.0001	–
Headache	4.3	9.1	0.08	2.2	7.9	0.002	–	4.9	10.5	–	2.6	2.9	0.004	–
Fatigue	20.4	25.2	–	8	16.4	0.04	–	19.8	23.2	–	12.3	19.3	0.05	–
Nausea	2.2	1.7	–	1.4	3	–	–	1.9	2.6	–	0.9	3.7	0.01	–
Dizziness	2.8	5.3	0.06	2	5.5	0.09	–	2.4	4.3	–	0.8	6.5	0.001	–
Feeling of intoxication	1.3	7.4	0.0005	1.4	5.4	0.01	–	1.5	5.9	0.0006	1.2	4.4	0.006	–

*Ratings in mm on a 0–100 mm visual analogue scale (VAS).
†Wilcoxon signed rank test, p value only given when <0.1.
‡Mann-Whitney U test, p value only given when <0.1.

Table 5 Percentage change in response to objective tests after two hours exposure to 150 ppm 2-propanol (2P)*

	0 h after exposure							3 h after exposure						
	Women (n=28)			Men (n=28)				Women (n=28)			Men (n=28)			
Test	Clean air	2P	p value, 2P *v* clean air†	Clean air	2P	p value, 2P *v* clean air†	p value, women *v* men‡	Clean air	2P	p value, 2P *v* clean air†	Clean air	2P	p value, 2P *v* clean air†	p value, women *v* men‡
Colour confusion index§	0.22	1.31	–	1.79	5.97	–	–	–2.63	9.49	–	–1.47	4.57	–	–
Pulmonary function														
VC	0.12	–0.20	–	–0.48	–0.03	–	–	–0.18	–0.91	–	–0.79	–0.49	–	–
FVC	0.82	–0.58	–	–0.43	–0.52	–	–	–0.06	–1.40	–	–0.38	–0.31	–	–
FEV_1	0.55	–0.03	–	0.54	–0.37	–	–	–0.53	–1.44	–	–0.13	–0.29	–	–
FEV_1/VC	0.41	0.20	–	1.12	–0.04	–	–	–0.44	–0.54	–	0.76	0.23	–	–
FEV_1/FVC	–0.22	0.66	–	0.46	0.11	–	–	–0.34	0.00	–	–0.20	0.05	–	–
FEF_{25}	1.08	1.58	–	2.08	0.18	–	–	–0.19	–0.38	–	1.82	–1.12	–	–
FEF_{50}	1.92	0.43	–	1.63	–1.17	–	–	1.12	–1.46	–	1.21	–0.81	–	–
FEF_{75}	–0.39	4.01	–	1.64	1.55	–	–	–5.53	–0.68	–	–2.26	1.64	–	–
PEF	–1.29	–0.17	–	–0.44	–0.75	–	–	–1.15	0.81	–	–0.66	–1.61	–	–
DL_{CO}	1.37	1.74	–	1.94	6.04	–	–	–	–	–	–	–	–	–
Nasal acoustic rhinometry														
Minimal cross sectional area	–3.45	–6.60	–	–3.34	0.93	–	–	–7.48	–8.30	–	–3.39	–0.40	–	–
Volume	–5.34	–9.98	–	–10.0	–7.00	–	0.09	–9.24	–12.0	–	–9.55	–6.09	–	–
Inflammatory markers in nasal lavage														
Lysozyme	n.a.¶	n.a.		n.a.	n.a.			32.4	35.4	–	17.3	12.7	–	–
Eosinophilic cationic protein	n.a.	n.a.		n.a.	n.a.			0.95	4.21	–	–1.32	–20.3	–	–
Myeloperoxidase	n.a.	n.a.		n.a.	n.a.			4.62	49.8	–	–1.55	0.63	–	–
Albumin	n.a.	n.a.		n.a.	n.a.			1.70	20.69	–	7.11	–4.54	–	–

*Calculated as the percentage change at 0 h and 3 h after exposure, respectively, compared to before exposure.
†Student's paired *t* test, p value only given when <0.1.
‡Student's *t* test, p value only given when <0.1.
§One male with congenital colour blindness excluded.
¶n.a., not analysed.

4). Correlation between rating of smell and other ratings were tested using the Spearman rank correlation test in StatView.

Prior to statistical analyses of the objective tests the values were recalculated as percentage change in response after exposure compared to before exposure. The Student's paired and unpaired *t* tests in StatView were used to test for differences in objective tests between exposure conditions and sexes, respectively (tables 5 and 6).

Table 6 Percentage change in response to objective tests after two hours exposure to 50 ppm *m*-xylene (mX)*

	0 h after exposure							3 h after exposure						
	Women (n=28)			Men (n=28)				Women (n=28)			Men (n=28)			
Test	Clean air	mX	p value, mX *v* clean air†	Clean air	mX	p value, mX *v* clean air†	p value, women *v* men‡	Clean air	mX	p value, mX *v* clean air†	Clean air	mX	p value, mX *v* clean air†	p value, women *v* men‡
Colour confusion index§	0.22	–3.26	–	1.79	3.78	–	–	–2.63	–4.49	–	–1.47	7.78	–	0.09
Pulmonary function														
VC	0.12	0.02	–	–0.48	0.40	–	–	–0.18	–1.66	–	–0.79	–0.77	–	–
FVC	0.82	0.40	–	–0.43	–0.49	–	–	–0.06	–2.81	0.01	–0.38	–0.76	–	0.05
FEV_1	0.55	0.83	–	0.54	0.28	–	–	–0.53	–1.73	–	–0.13	0.05	–	–
FEV_1/VC	0.41	0.82	–	1.12	–0.07	–	–	–0.44	–0.03	–	0.76	0.71	–	–
FEV_1/FVC	–0.22	0.43	–	0.46	0.78	–	–	–0.34	1.09	0.03	–0.20	0.80	–	–
FEF_{25}	1.08	2.49	–	2.08	–1.79	–	–	–0.19	0.70	–	1.82	–1.84	–	–
FEF_{50}	1.92	2.95	–	1.63	0.20	–	–	1.12	1.48	–	1.21	3.39	–	–
FEF_{75}	–0.39	2.46	–	1.64	6.88	–	–	–5.53	3.32	0.04	–2.26	6.43	–	–
PEF	–1.29	2.38	–	–0.44	–2.78	–	–	–1.15	0.75	–	–0.66	–4.13	–	–
DL_{CO}	1.37	5.93	–	1.94	–0.47	–	–	–	–	–	–	–	–	–
Nasal acoustic rhinometry														
Minimal cross sectional area	–3.45	1.48	–	–3.34	2.93	–	–	–7.48	–3.96	–	–3.39	1.58	–	–
Volume	–5.34	–5.04	–	–10.0	–9.05	–	–	–9.24	–7.07	–	–9.55	–7.81	–	–
Inflammatory markers in nasal lavage														
Lysozyme	n.a.¶	n.a.		n.a.	n.a.			32.4	33.2	–	17.3	15.3	–	–
Eosinophilic cationic protein	n.a.	n.a.		n.a.	n.a.			0.95	1.14	–	–1.32	2.83	–	–
Myeloperoxidase	n.a.	n.a.		n.a.	n.a.			4.62	51.2	–	–1.55	0.79	–	–
Albumin	n.a.	n.a.		n.a.	n.a.			1.70	36.1	–	7.11	4.24	–	–

*Calculated as the percentage change at 0 h and 3 h after exposure, respectively, compared to before exposure.
†Student's paired *t* test, p value only given when <0.1.
‡Student's *t* test, p value only given when <0.1.
§One male with congenital colour blindness excluded.
¶n.a., not analysed.

Table 7 Findings of effect responses by analysis of variance*

Effect variable	Time	Exposure	Sex	Time × exposure	Time × sex	Exposure × sex	Time × exposure × sex
Blinking frequency	0.002	–	0.09	–	–	–	–
Color confusion index	–	–	0.099	–	–	–	–
Pulmonary function							
VC	0.01	–	–	–	–	–	–
FVC	0.02	–	–	0.09	0.02	–	–
FEV_1	0.005	–	–	–	0.04	–	–
FEV_1/VC	–	–	–	–	0.06	–	–
FEV_1/FVC	–	–	–	–	–	–	–
FEF_{25}	–	–	–	–	–	–	–
FEF_{50}	–	–	–	–	–	–	–
FEF_{75}	–	0.08	–	–	–	–	–
PEF	–	–	–	–	–	–	–
DL_{CO}	–	–	–	–	–	0.04	–
Nasal measurements							
Minimal cross sectional area	0.06	–	0.09	–	–	–	–
Volume	–	–	–	–	0.05	–	–

Effect response is the relative change during or after solvent exposure compared to prior exposure.
*Repeated measures ANOVA, p values only given when <0.1.

The percentage changes in the objective tests were also analysed by repeated measures analysis of variance (ANOVA) using the StatView software. In the ANOVA analyses, for each response variable several factors were addressed in parallel, namely: does the response change over the day ("time")?; is the response different in size for different exposure conditions ("exposure")?; is the response different in size for women and men ("sex")?; does the response change over the day, in different ways for different exposure conditions ("time × exposure")?; does the response change differently for women and men over the day ("time × sex")?; does the response change differently for women and men, in different ways for different exposure conditions ("exposure × sex")?; and does the response change differently for women and men over the day, in different ways for different exposure conditions ("time × sex × exposure")? (table 7).

In all statistical analyses the significance level was set at 0.05. However, all p values below 0.10 were tabulated to assist in identifying tendencies.

RESULTS

Symptom ratings

Independent of exposure, women tended to rate symptoms slightly higher than men (table 2).

In both sexes, nearly all symptom ratings (the only exceptions being "nausea" in women after 60 minutes of exposure to 2-propanol and *m*-xylene, respectively) increased during solvent exposure, compared to control exposure. These increases were significant ($p < 0.05$, Wilcoxon signed rank test) for most symptoms at at least one of the two time points 60 minutes and 118 minutes. However, the average ratings during solvent exposure did not exceed that corresponding to "somewhat" on the VAS scale (tables 3 and 4). Considering differences in response between sexes, the rating of "discomfort in the throat or airways" was significantly increased in women after 60 minutes of exposure to both 2-propanol ($p = 0.04$) and *m*-xylene ($p = 0.02$). The rating of "fatigue" was increased more in men after one hour ($p = 0.02$) but increased more in women after two hours ($p = 0.04$) of exposure to *m*-xylene. This rating also increased markedly among females during control exposure (tables 3 and 4).

At all time points during exposure to solvent, there was only a weak correlation between the rating of smell and other ratings, with rho values ranging between 0.04 and 0.41 in the Spearman rank correlation test (data not shown). This suggests that the perception of exposure did not in it self heavily influence the magnitude of symptoms rating.

Pulmonary function

No significant effects of solvent exposure were seen on pulmonary function in men. In women, 2-propanol had no effect, whereas FVC was decreased and FEV_1/FVC and FEF_{75} were increased three hours after exposure to *m*-xylene. The only significant sex difference in response was that women had a more marked decrease in FVC than men three hours after exposure to *m*-xylene (tables 5 and 6).

In the ANOVA, significant effects of time and time × sex were seen for FEV_1 and FVC. Together with visual inspection of the data (not shown), this indicates that, independent of the exposure condition, there is a change in lung function throughout the day and that this change is more pronounced in women (table 7).

With respect to lung diffusing capacity for carbon monoxide, there was a significant sex difference in response between exposure conditions in the ANOVA ($p = 0.4$, table 7). Visual inspection of the data (not shown) suggests an increase in DL_{CO} in women after exposure to *m*-xylene and an increase in men after exposure to 2-propanol. However, none of these changes were significant in the *t* tests.

Acoustic rhinometry

There were no significant effects of solvent exposure on nasal volume or cross sectional area (tables 5 and 6). However, the ANOVA analysis indicated a sex dependent decrease in nasal volume over time ($p = 0.05$, table 7).

Inflammatory markers

There were no significant differences between sexes or effects of exposure to either 2-propanol or *m*-xylene on inflammatory markers in nasal lavage. The average levels of myeloperoxidase and albumin increased by about 20–50% in women exposed to 2-propanol or *m*-xylene. However, this is probaby a chance phenomenon because of the high variability in marker concentrations (tables 5 and 6).

Blinking frequency

There were no significant differences between sexes or effects of exposure on blinking frequency. The ANOVA revealed a change in frequency over time ($p = 0.002$, table 7). This can be explained by a higher blinking frequency during the first minutes of exposure, which may have been caused by increased alertness as a result of entering a new environment (fig 3).

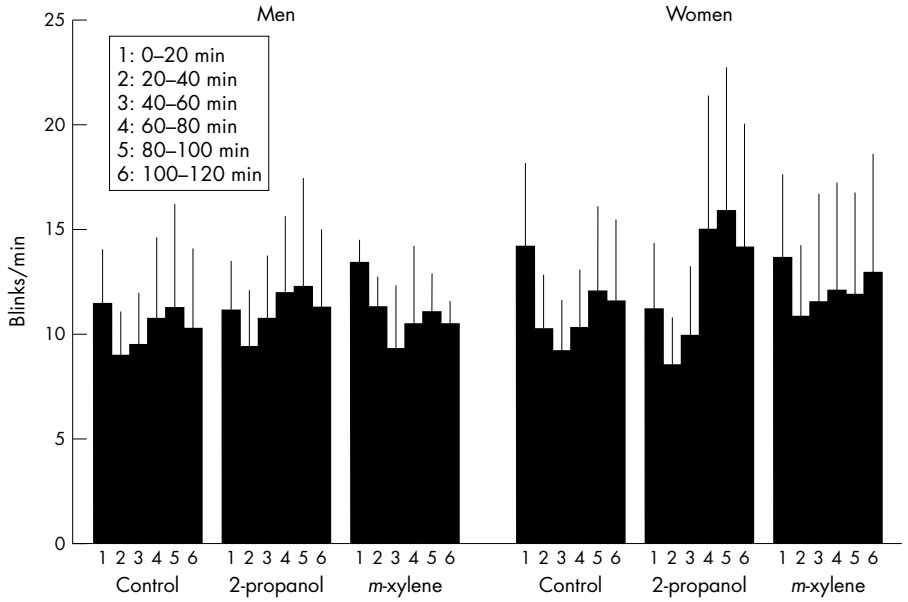

Figure 3 Average blinking frequency in men and women during exposure to clean air, 150 ppm 2-propanol, or 50 ppm *m*-xylene. Results are presented in time series as blinks per 20 minutes. The upper 95% confidence limits (vertical lines) are indicated to illustrate the variability in responses.

Colour vision

Unexposed women performed better in the colour vision test (that is, lower CCI) than unexposed men ($p = 0.04$, table 2). This is also reflected by a tendency of sex dependent difference in CCI response in the ANOVA analysis ($p = 0.099$, table 7). Furthermore, there were non-significant tendencies of increased CCI in both women and men after exposure to 2-propanol and in men after exposure to *m*-xylene compared to clean air. Women exposed to *m*-xylene, however, tended to improve their colour vision (tables 5 and 6).

DISCUSSION

In this study we attempted to test the hypothesis that women and men differ in their sensitivity to the acute effects of two common solvents, 2-propanol and *m*-xylene. Ethical considerations and experimental costs limit exposure levels and number of subjects that can be studied, and thus reduce the power to detect any sex differences if they exist. Nevertheless, we were able to show some interesting findings. Unexposed women tended to rate symptoms slightly higher than men and performed better in the colour vision test. On exposure to 2-propanol or *m*-xylene vapours women rated irritative symptoms higher than men. A more doubtful finding was that some lung function parameters were affected in women at three hours after exposure to *m*-xylene. In particular, FVC decreased more in women than in men at this point.

One strength in a study of this kind is that volunteers are exposed to solvents under well controlled conditions, with respect to solvent exposure level and duration as well as temperature and humidity. A further advantage is that the subjects served as their own controls to exclude spurious effects caused by individual differences in physiological parameters. To reduce the risks for selection bias, subjects in the present study were recruited from an original group of 2000 citizens randomly selected from the Stockholm County Council population. Judging by age, the sex selection bias was minimal (participating women 34.3 years, participating men 34.5, versus recruitment population 35.0 years). A problem with this approach, as with any recruitment process, is that one may expect an under representation of individuals who dislike the smell or have other negative experiences of solvents and chemicals in general. Thus, there is a possibility that, because of self selection, the participating women to a larger (or lesser) extent than the participating men were recruited from "non-afraid" subgroups of the population. If this is the case, a true or marked sex difference in solvent sensitivity may be masked or reduced.

The response rate in the recruitment was low and 73% of those who initially agreed participation turned out to be non-participants (fig 1). Possible explanations of the low response rate and high percentage of drop outs are: a "fear of chemicals", a negative attitude towards experiments with volunteers and/or exposure chambers, insufficient economic compensation, and lack of time. The last explanation is especially obvious for those employed on a regular Monday to Friday basis, as the experiment involved three whole days from 08 00 to 16 00. Among the 56 participants, nine women (33% of the females) and 14 men (50% of the males) were students. In the Stockholm population the proportion of students is 14% of the women and 8% of the men. Thus, there was a heavy over representation of students, twofold for women and sixfold for men, compared to the general population. Furthermore, of the participants, five women (18% of the females) and six men (21% of the males) were shift workers. This is almost the same as in the general population where the proportion of shift workers is 24% of the women and 19% of the men.[1]

The order of exposure may influence the measured effects, especially self reported symptoms. The same is true for the presence of other persons in the chamber. We therefore originally tried to balance the exposure orders and have the same number (2+2) of women and men on every exposure occasion. However, for different reasons, some volunteers postponed their participation, thereby disturbing the exposure scheme. The statistical analyses revealed no significant difference in exposure pattern between sexes and no significant influence of exposure order on responses. Thus, although the possibility that the design imbalances may have influenced the results cannot be ruled out, we conclude that they did at least not have a major impact on the outcome of the study.

The result of the average ratings prior to exposure (table 2) suggests that women tend to rate higher intensities than men. This is supported by a study by Tibblin and colleagues,[25] who investigated the occurrence of 30 symptoms by age and sex in two population studies and found that women in general rated symptoms like dizziness, headache, general fatigue, and

nausea higher. These authors suggested that women and men have different lifestyles, and that women's higher degree of symptoms may be a result of more responsibilities and higher workload (job, home, and bringing up the children). On the other hand, Pennebaker[26] noticed a sex difference in how individuals notice, define, and react to symptoms in that women are particularly sensitive to external environmental signs in defining their symptoms while men are more attentive to bodily changes. One explanation of the higher symptom ratings among women in our present study could thus be that women expect to be exposed to a stressful and potentially toxic environment.

In the present study we saw significantly increased ratings for nearly all symptoms following solvent exposure compared to clean air exposure. In a study by Muttray and colleagues,[27] a group of 24 healthy men were exposed to a higher level of 886 mg/m^3 (380 ppm) 2-propanol in an exposure chamber. The symptoms studied were related to discomfort, tiredness, irritation, and breathing difficulties. After two and four hours of exposure the smell of solvent was significantly more pronounced, but no other effects were observed. In a study of toluene (400 mg/m^3, 6.5 hours), an aromatic hydrocarbon similar to *m*-xylene, irritation of eyes, nose, and throat was significantly increased in comparison to clean air exposure.[28] In another study, the ratings of discomfort in throat or airways were significantly increased during exposure to toluene combined with chlorzoxazone (200 mg/m^3, 2 h + 500 mg, respectively), and the same tendency, although not significant, was seen during exposure to toluene alone in a study by Ernstgård and colleagues.[29] The different outcomes may be caused by differences in exposure levels, number of exposed subject (power), and/or how volunteers were recruited and selected.

In the present study, a small effect on the lung function in women exposed to *m*-xylene was detected. We also noted a change in lung function over the day, this change being more pronounced in women. Diurnal variation in lung function and diffusing capacity have been reported by several investigators.[30–32] In a large study with 876 subjects, no differences in diurnal variability were seen between men and women.[30] In a study in rats by Massaro and colleagues,[33] the authors concluded that oestrogen induces smaller and more numerous alveoli, resulting in a sexual dimorphism of the gas exchange region in the lungs. Oestrogen may be a contributing factor to the subtle sex differences in pulmonary function in our present study. Another explanation might be that, on average, women are smaller than men and have smaller airways which are more sensitive to a swelling. According to Sparrow and colleagues,[34] there is an association between airway responsiveness and a low FEV_1.

Our study also indicates a tendency to a sex difference in the decrease of nasal volume during the day with the females having a larger percentual decrease. This tendency was seen at all three exposure conditions including clean air. In addition, a tendency to a small sex difference in minimal cross sectional area was seen, an observation also made by Millqvist and Bende.[35] A possible explanation is that the females have a smaller nasal cavity than the males, and that the same degree of mucosal swelling thus causes a greater percentual decrease in volume and area in women.

By measuring eye blinks with electromyography (EMG), we could get an objective indicator of the eye irritation. Eye blinking is a normal way to protect the eye against dehydration, irritants, and mechanical damage. Air pollution may cause eye irritation, either directly by stimulating the trigeminal nerve, or indirectly by a reduction of tear film stability. Thus, increased blinking may be an early marker of eye irritation from exposure to irritants caused by air pollutants. During the second hour of exposure to 2-propanol there was a tendency to an increased blinking frequency in females (not significant). This is in line with the increased rating of irritative symptoms in women exposed to 2-propanol. At all exposure conditions the blinking frequency was increased, during the first minutes in the exposure chamber. This was probably caused by increased alertness and could be considered an "adaptation phase".

We found a significantly higher CCI among unexposed men. Subjects with known congenital colour vision defects were excluded and there was no bimodal distribution in CCI, which would indicate subclinical colour blindness. Thus, the well known higher prevalence of colour blindness among men cannot explain our finding. To our knowledge, this is the first study indicating that women may have a better colour vision than men.

In a study of this kind, the volunteers can easily detect the exposure condition by smell; blinding is therefore not possible. However, correlations between ratings of smell and rating of symptoms were weak. Thus, it seems unlikely that the perceived exposure by itself severely affected the symptom ratings.

Using the subjects as their own controls, we were able to show subtle effects of solvent exposure on the rating of irrative symptoms. To detect sex differences in response would require a larger number of subjects, since comparisons must be made on a group basis. The possibility to detect true differences between groups obviously varies widely for different effect parameters. A power analysis using the observed standard deviations in our present study indicates that, with the given sample size (28 subjects per sex), the likelihood to detect a 5% sex difference in response is, for example, 0.999 for FEV_1, 0.34 for nasal volume, and 0.054 for myeloperoxidase in nasal lavage. Expressed in different terms, true differences in response between sexes of about 3% for FEV_1, 9% for nasal volume, and 75% for myeloperoxidase in nasal lavage are needed to achieve an acceptable power (0.8) to detect the differences.

One possible explanation of the observed sex differences is a difference in toxicokinetic behaviour, so that women on average experience a different internal exposure than men, given identical external solvent exposure levels. We are undertaking a follow up study to address the issue of toxicokinetic differences using a similar experimental design (two hour chamber exposure to 2-propanol and *m*-xylene, results to be published).

In conclusion, our results suggest that women are slightly more sensitive than men to the irritative effects of 2-propanol and *m*-xylene and probably to solvent vapours in general. Further studies are needed to confirm or reject this suggestion.

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