eTable 1: Table describing the number of cases with major congenital malformations by organ system, overall, among those who had their urinary metabolites determined, and among those diagnosed during the 2-year follow-up

	Major Malformations (Total cohort N=3438[a])	Major Malformations With Alkoxycarboxylic Acids Analyzed	Major Malformations With Chlorinated Metabolites Analyzed	Additional Major Malformations Diagnosed during the 2-year Follow up (N=1505)
Organ System	No.	No.	No.	No.
Central nervous system	5	4	3	
Eye	1	1	1	
Cardiac	6	5	5	4
Orofacial	8	8	4	
Digestive	2	2	2	
Abdominal wall	1	1	1	
Respiratory	1	1	0	
Urinary tract	15	14	11	2
Male genital	12	12	8	
Limb	22	22	15	1
Musculoskeletal	2	2	1	
Cutaneous	4	4	3	
Chromosomal anomalies/Genetic syndromes[c]	18	-	-	
TOTAL	94[b]	79[b]	51[b]	7

[a]3438 children or fetuses including livebirths, stillbirths, and terminations of pregnancy, twin pregnancies included

[b] 1 live birth and 2 elective abortions had two major malformations each

[c] 14 chromosomal anomalies: 10 Down syndrome, 1 Edwards syndrome, 1 Trisomy 13, 1 Turner syndrome, 1 Klinefelter syndrome; 4 genetic syndromes: 1 restrictive dermopathy syndrome, 1 Goldenhar syndrome, 1 Pierre Robin syndrome, 1 branchio-oto-renal syndrome

eAppendix 1: Appendix describing the procedures for collection, transport, storage, and chemical analysis of urine samples and for the study of their validity

a) Description of urine collection procedure and duration of the different steps

Biomonitoring of urinary metabolites of glycol ethers has been recommended for the surveillance of occupational exposures since 1984 (Smallwood AW et al. 1984) and that of chlorinated solvents since 1951 (Ahlmark A & Forssman S 1951). No particular procedure or vial material (glass or plastic) has been recommended, for no interference has ever been reported.

In our study, each participating physician regularly received sampling kits to be distributed to participating women. The vials were prepared beforehand at the laboratory, with acidic drops added to reach a concentration of 1 mM. This addition of acid was intended to reduce bacterial proliferation in view of the time urine samples were expected to be at room temperature during transportation, before arrival at the laboratory. This acidification procedure has also been shown to improve the efficiency of the extraction phase of chemical analytical procedures (Shih TS et al. 1999).
The recruiting physicians gave each woman 2 polypropylene vials (10 mL each) with instructions for urine collection, specifically, that urine be collected first thing in the morning, that both vials be filled, put in the shipping box provided, and sent by regular mail to the laboratory together with the questionnaire (which included the sampling date). On arrival at the lab, vials were frozen at -20°C until analysis.

The median transportation time from the participants` homes to the laboratory was 2 days [10^{th} percentile=1 day; 90^{th} percentile=5 days]. The median duration of storage at -20°C until chemical analysis was 3.00 years ([10^{th} percentile=1.85 years; 90^{th} percentile=4.70 years].

Ahlmark A, Forssman S. Evaluating trichloroethylene exposure by urine analyses for trichloroacetic acid, Ind. Hyg. Occup. Med., 1951, 3, 386-398
Shih TS et al. Improved method to measure urinary alkoxyacetic acids. Occup Environ Med. 1999, 56:460-7
Smallwood AW et al. Analyses of ethylene glycol monoalkyl ethers and their proposed metabolites in blood and urine. Environ Health Perspect. 1984 57: 249-53

b) Available published information on stability of metabolites in time

Prolonged storage of urine samples at room temperature (20°C) for up to 30 days has been reported to have no effect on the level of urinary TCAA (Antczak K. 1989).
Similarly the storage of urine samples at -20°C for up to 8 months was reported to have no effect on levels of alkoxyacetic acids (Smallwood AW et al. 1988; Shih et al. 1999).

Antczak K. Evaluation of the stability of biological specimens used for monitoring of occupational exposure. Studies of the stability of phenol and trichloroacetic acid in urine. Med Pr. 1989;40:362-8
Shih TS et al. Improved method to measure urinary alkoxyacetic acids. Occup Environ Med. 1999, 56:460-7

Smallwood AW et al. Determination of urinary 2-ethoxyacetic acid as an indicator of occupational exposure to 2-ethoxyethanol. Appl Ind Hyg 1988, 3: 47–50

c) Chemical analysis of metabolites in urine

Chemical analyses, blinded to case-control status were performed at the Toxicology and Genopathy Laboratory in Lille Hospital (France). GE metabolites were measured with high-resolution gas chromatography coupled to mass spectrometry detection (GC-MS), as described in Labat et al. (2008). This method can simultaneously analyze 8 alkoxycarboxylic acid metabolites, 7 alkoxyacetic acids from ethylene glycol ether derivatives: methoxyacetic acid (MAA, mainly derived from ethylene glycol methyl ether, i.e., EGME), ethoxyacetic acid (EAA, mainly derived from ethylene glycol ethyl ether, i.e., EGEE), butoxyacetic acid (BAA, mainly derived from ethylene glycol butyl ether, i.e., EGBE), n-propoxyacetic acid (PAA, derived from ethylene glycol n-propyl ether), phenoxyacetic acid (PhAA, derived from ethylene glycol phenyl ether, i.e., EGPhE), methoxy ethoxyacetic acid (MEAA mainly derived from diethylene glycol methyl ether, i.e., DEGME), ethoxy ethoxyacetic acid (EEAA mainly derived from diethylene glycol ethyl ether, i.e., DEGEE), and one alkoxyproprionic acid from a propylene glycol ether derivative: 2-methoxypropionic acid (2-MPA, derived from the minor isomer of propylene glycol methyl ether, i.e., PGME). The detection limit (LOD) was 0.05 mg/L for each alkoxycarboxylic acid. TCAA and TCOH were assessed by GC-MS, as described by Dehon et al. (2000), with a LOD equal to 0.01 mg/L for both compounds.

Labat L, Humbert L, Dehon B, Multigner L, Garlantezec R, Nisse C et al. Dosage des métabolites urinaires des éthers de glycol par chromatographie en phase gazeuse couplée à la spectrométrie de masse [in French]. Ann Toxicol Anal. 2008;20:227-32
Dehon B, Humbert L, Devisme L, Stievenart M, Mathieu D, Houdret N et al. Tetrachloroethylene and trichloroethylene fatality: Case report and Simple Headspace SPME-capillary gas chromatographic determination in tissues. J Anal Toxicol. 2000;24:22-6

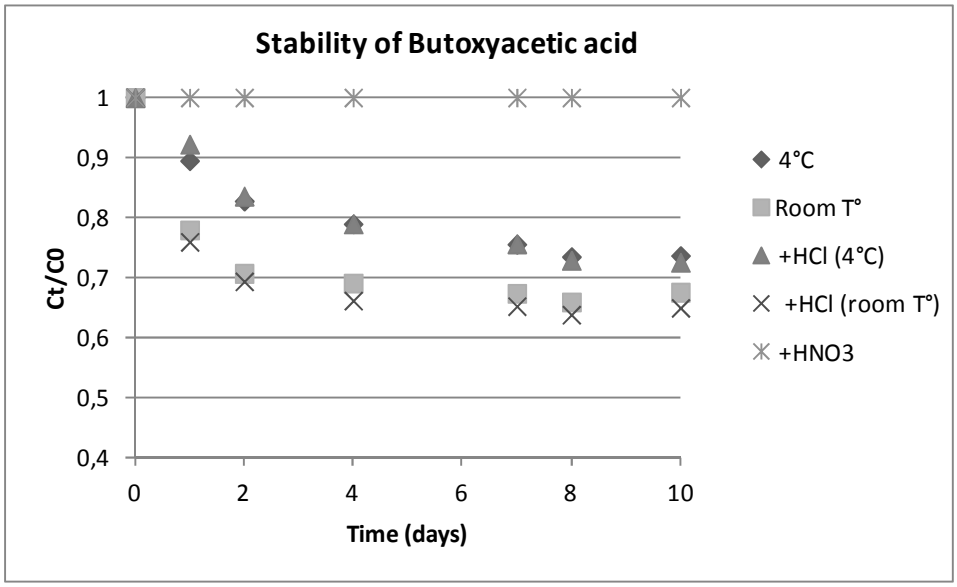
d) Results of the experiment conducted to assess the stability of acidic metabolites according to duration of transport at room temperature and addition of acidic stabilizer

The stability of 6 ester acetic acids (PhAA, MEAA, EAA, TCAA, MAA, BAA) was been evaluated by considering temperature and time of preservation. Aqueous solutions of each compound were tested for 10 days at room temperature and at 4°C in the presence and absence of HNO_3 or HCl (at a concentration of 1 mM). Concentrations in the range of mg/mL were used and monitored by UV spectrophotometry. Experiments were performed in triplicate.

PhAA, MEAA, EAA, TCAA and MAA showed very good stability for 10 days even at room temperature and without the addition of any preservatives. The table below shows the average recovery of the substances. In view of the precision of the measurement, the solutions can be considered stable. Solutions containing HNO_3 and HCl were also tested and showed no changes over time at either 4°C or at room temperature.

	Tested concentration (mg/mL)	Average of Ct/C0 at day 10	
		Room T°C	4°C
PhAA	0.1	1.03	1.02
MEAA	1.1	1.06	0.95
EAA	1.1	0.97	1.03
TCAA	1.0	0.98	0.96
MAA	1.0	1.02	1.02

The results showed that BAA behaved differently than the other compounds. At room temperature, more than 30% of the BAA was lost without stabilizer and in the presence of HCl. Reducing the temperature (from room temperature to 4°C) improved initial stability, but after 10 days, a loss around 30% was again observed. The addition of HNO_3 stabilized the solution at both room temperature and 4°C..



Finally, urine samples were spiked with PhAA and monitored for 10 days at room temperature and 4°C. Urine samples without spiking were used as blanks. The comparison of the two samples showed that PhAA was stable at either temperature, with or without preservative.

e) A posteriori study of the influence of the sampling conditions on urinary levels of biomarkers

We studied the influence of sampling conditions (urinary creatinine level, gestational age at inclusion (<10, 10-13, >13 weeks), type of stabilizer, transportation time at room temperature (1 day, 2-3 days and 4 days and more) and duration of storage before analysis) on the probability of detection and the levels of the urinary metabolites. Different multivariate models were used according to the percentage of detection (Helsel et al., 2002; Lubin et al., 2004). Metabolites identified in more than half of the samples were analyzed as continuous variables. Multiple tobit regression was used with log-transformed metabolite values as the dependent variable, censored at the LOD. Beta coefficients are presented with their 95% CIs.

Metabolites identified in fewer than half of the subjects were defined in two classes (<LOD vs ≥LOD). ORs and their 95% CIs were used to express the relations between metabolites that were found versus not found. The models presented here are adjusted for the 5 covariates (creatinine level, gestational age at inclusion, type of stabilizer, transportation time at room temperature, and storage duration between collection and analysis) and for self-report of exposure to products that may contain solvents.

Creatinine level was associated with EEAA, BAA and PhAA. Type of stabilizer was associated with level of MEAA. Transportation time appeared to influence the levels of BAA, and the detection of 2-MPA and TCOH. Duration of storage was associated with levels of MEAA, EEAA, PAA and 2-MPA.

These covariates (creatinine level, gestational age at inclusion, transportation time, and storage duration between collection and analysis) were therefore studied as potential confounders of the OR estimates of the risk of malformations associated with the urinary level of each metabolite.

Helsel DR, Hirsch M. Methods for data below the reporting Limit. In : Techniques of Water-Resources Investigations of the United States Geological Survey. Book 4, Hydrologic Analysis and Interpretation. Chapter A3 Statistical Methods. Eds : U.S. Geological Survey. 2002. (available at http://www.practicalstats.com/aes/aes/AESbook_files/HelselHirsch.PDF).
Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, Bernstein L, Hartge P. Epidemiologic evaluation of measurement data in the presence of detection limits. Environ Health Perspect. 2004 Dec;112(17):1691-6.

Table 1.1: Relation between metabolite detection and physiological or sampling collection characteristics

		MAA							MEAA			
		<LOD n	%	>=LOD n	%	OR[a]	[95% CI]]		GM (mg/L)	beta[a]	[95% CI]	
Urinary creatinine level	<0.82	104	25.2	33	19.8	Ref			0.07	Ref		
	[0.82-1.13[	107	25.9	42	25.2	1.27	0.74	2.19	0.08	-0.07	-0.36	0.22
	[1.13-1.46[	99	24.0	42	25.2	1.45	0.84	2.51	0.10	0.08	-0.22	0.37
	>=1.46	103	24.9	50	29.9	1.64	0.96	2.80	0.10	0.04	-0.25	0.33
Gestational age at inclusion (weeks)												
	<10	101	24.5	43	25.8	Ref			0.07	Ref		
	[10-13]	195	47.2	77	46.1	1.00	0.63	1.58	0.09	0.14	-0.12	0.40
	>13	117	28.3	47	28.1	1.04	0.63	1.73	0.10	0.26	-0.01	0.54
Type of stabilizer	HNO3-	334	80.9	125	74.9	Ref			0.11	Ref		
	HCL	79	19.1	42	25.1	2.06	0.76	5.64	0.03	-1.25	-1.77	-0.74
Transportation time (days)	1	183	44.4	63	38.2	Ref			0.09	Ref		
	2 or 3	140	34.0	67	40.6	1.37	0.90	2.08	0.08	-0.06	-0.28	0.17
	>=4	89	21.6	35	21.2	1.10	0.68	1.80	0.08	-0.13	-0.39	0.14
Storage duration in days (between collection and analysis)	<865	108	26.2	37	22.2	Ref			0.11	Ref		
	[865-1096[	104	25.2	41	24.6	1.13	0.67	1.92	0.14	0.35	0.06	0.64
	[1096-1376[	103	24.9	42	25.2	1.14	0.68	1.95	0.10	0.00	-0.28	0.29
	>1376	98	23.7	47	28.1	0.78	0.29	2.15	0.04	-0.02	-0.53	0.49

GM: geometric means. beta : beta coefficient of the tobit regression of the logarithm of the metabolites under study.

[a] adjusted for urinary creatinine level, gestational age at inclusion, type of stabilizer, transportation time, storage duration and self-report of exposure to products that may contain solvents

Table 1.1 (continued): Relation between metabolite detection and physiological or sampling collection characteristics

		EAA							EEAA						
		<LOD		>=LOD		OR[a]	[95% CI]		<LOD		>=LOD		OR[a]	[95% CI]	
		n	%	n	%				n	%	n	%			
Urinary creatinine level	<0.82	135	24.2	2	8.7	Ref			125	26.3	12	11.4	Ref		
	[0.82-1.13[	143	25.7	6	26.1	2.33	0.45	12.14	134	28.2	15	14.3	1.29	0.57	2.90
	[1.13-1.46[	131	23.5	10	43.5	4.57	0.95	22.03	114	24.0	27	25.7	2.83	1.34	5.98
	>=1.46	148	26.6	5	21.7	1.92	0.36	10.40	102	21.5	51	48.6	6.58	3.24	13.35
Gestational age at inclusion (weeks)	<10	136	24.4	8	34.8	Ref			120	25.3	24	22.9	Ref		
	[10-13]	261	46.9	11	47.8	0.68	0.26	1.80	219	46.1	53	50.5	1.38	0.78	2.42
	>13	160	28.7	4	17.4	0.36	0.10	1.29	136	28.6	28	26.7	1.09	0.58	2.05
Type of stabilizer	HNO3-	437	78.5	22	95.7	Ref			384	80.8	75	71.4	Ref		
	HCL	120	21.5	1	4.3	0.17	0.01	2.99	91	19.2	30	28.6	1.66	0.55	5.05
Transportation time (days)	1	231	41.7	15	65.2	Ref			198	42.0	48	45.7	Ref		
	2 or 3	202	36.5	5	21.7	0.41	0.14	1.17	174	36.9	33	31.4	0.70	0.42	1.19
	>=4	121	21.8	3	13.0	0.35	0.10	1.24	100	21.2	24	22.9	0.93	0.52	1.65
Storage duration in days (between collection and analysis)															
	<865	138	24.8	7	30.4	Ref			119	25.1	26	24.8	Ref		
	[865-1096[	136	24.4	9	39.1	1.12	0.40	3.18	121	25.5	24	22.9	0.95	0.50	1.81
	[1096-1376[	140	25.1	5	21.7	0.63	0.19	2.09	125	26.3	20	19.1	0.76	0.39	1.48
	>1376	143	25.7	2	8.7	0.90	0.10	7.91	110	23.2	35	33.3	1.34	0.44	4.07

[a] adjusted for urinary creatinine level, gestational age at inclusion, type of stabilizer, transportation time, storage duration and self-report of exposure to products that may contain solvents

[b] adjusted for urinary creatinine level, gestational age at inclusion, transportation time, storage duration and self-report of exposure to products that may contain solvents

Table 1.1 (continued): Relation between metabolites detection and physiological or sampling collection characteristics

		PAA							BAA[c]			
		<LOD		>=LOD		OR[a]	[95% CI]		GM (mg/l)	beta[b]	[95% CI]	
		n	%	n	%							
Urinary creatinine level	<0.82	124	23.9	13	21.0	Ref			0.09	Ref		
	[0.82-1.13[	130	25.1	19	30.7	1.24	0.57	2.68	0.11	0.14	-0.03	0.31
	[1.13-1.46[	125	24.1	16	25.8	1.11	0.50	2.48	0.12	0.18	0.01	0.35
	>=1.46	139	26.8	14	22.6	0.80	0.35	1.80	0.12	0.18	0.01	0.34
Gestational age at inclusion (weeks)	<10	128	24.7	16	25.8	Ref			0.11	Ref		
	[10-13]	238	46.0	34	54.8	1.11	0.58	2.48	0.12	0.05	-0.09	0.20
	>13	152	29.3	12	19.4	0.62	0.50	1.42	0.10	-0.08	-0.24	0.08
Type of stabilizer	HNO3-	400	77.2	59	95.2	Ref			0.11			
	HCL	118	22.8	3	4.8	0.29	0.05	1.78	-			
Transportation time (days)	1	218	42.3	28	45.2	Ref			0.11	Ref		
	2 or 3	186	36.1	21	33.9	0.94	0.51	1.74	0.11	-0.06	-0.19	0.07
	>=4	111	21.6	13	21.0	0.96	0.47	1.96	0.10	-0.16	-0.31	-0.02
Storage duration in days (between collection and analysis)	<865	127	24.5	18	29.0	Ref			0.11	Ref		
	[865-1096[	126	24.3	19	30.7	1.00	0.50	2.01	0.10	-0.03	-0.17	0.11
	[1096-1376[	125	24.1	20	32.3	1.13	0.57	2.27	0.12	0.10	-0.04	0.25
	>1376	140	27.0	5	8.1	0.25	0.09	0.69	0.12	0.12	-0.14	0.38

GM: geometric means. beta: beta coefficient of the tobit regression of the logarithm of the metabolites under study

[a] adjusted for urinary creatinine level, gestational age at inclusion, type of stabilizer, transportation time, storage duration and self-report of exposure to products that may contain solvents

[b] adjusted for urinary creatinine level, gestational age at inclusion, transportation time, storage duration and self-report of exposure to products that may contain solvents; c samples with HNO3 stabilizer only

Table 1.1 (continued): Relation between metabolites detection and physiological or sampling collection characteristics

		PhAA				2-MPA						
		GM (mg/L)	beta[a]	[95% CI]		<LOD n	%	>=LOD n	%	OR[b]	[95% CI]	
Urinary creatinine level	<0.82	0.29	Ref			132	24.0	5	16.7	Ref		
	[0.82-1.13[	0.35	0.10	-0.23	0.42	143	26.0	6	20.0	0.94	0.27	3.23
	[1.13-1.46[	0.43	0.28	-0.05	0.62	133	24.2	8	26.7	1.42	0.43	4.62
	>=1.46	0.57	0.49	0.16	0.81	142	25.8	11	36.7	1.63	0.54	4.94
Gestational age at inclusion (weeks)	<10	0.38	Ref			136	24.7	8	26.7	Ref		
	[10-13]	0.38	0.09	-0.20	0.37	255	46.4	17	56.7	1.28	0.52	3.15
	>13	0.45	0.22	-0.09	0.53	159	28.9	5	16.7	0.56	0.17	1.80
Type of stabilizer	HNO3-	0.40	Ref			429	78.0	30	100.0	Ref		
	HCL	0.41	0.10	-0.48	0.68	121	22.0	0	0.0	-		
Transportation time (days)	1	0.38	Ref			239	43.7	7	23.3	Ref		
	2 or 3	0.41	-0.01	-0.27	0.25	195	35.7	12	40.0	2.20	0.84	5.77
	>=4	0.42	-0.07	-0.37	0.23	113	20.7	11	36.7	3.62	1.35	9.76
Storage duration in days (between collection and analysis)	<865	0.46	Ref			135	24.6	10	33.3	Ref		
	[865-1096[	0.38	-0.01	-0.33	0.31	136	24.7	9	30.0	0.84	0.33	2.19
	[1096-1376[	0.36	-0.17	-0.48	0.15	136	24.7	9	30.0	0.88	0.34	2.27
	>1376	0.41	-0.04	-0.61	0.53	143	26.0	2	6.7	0.18	0.04	0.85

GM: geometric means. beta: beta coefficient of the tobit regression of the logarithm of the metabolites under study

[a] adjusted for urinary creatinine level, gestational age at inclusion, type of stabilizer, transportation time, storage duration and self-report of exposure to products that may contain solvents

[b] adjusted for urinary creatinine level, gestational age at inclusion, transportation time, storage duration and self-report of exposure to products that may contain solvents

Table 1.1 (continued): Relation between metabolites detection and physiological or sampling collection characteristics

		TCAA[a]							TCOH[a]						
		<LOD		>=LOD		OR[b]	[95% CI]		<LOD		>=LOD		OR[b]	[95% CI]	
		n	%	n	%				n	%	n	%			
Urinary creatinine level	<0.82	88	20.7	8	24.2	Ref			93	21.5	3	11.1	Ref		
	[0.82-1.13[	113	26.5	5	15.2	0.50	0.16	1.61	113	26.2	5	18.5	1.32	0.30	5.87
	[1.13-1.46[	106	24.9	8	24.2	0.78	0.27	2.22	109	25.2	5	18.5	1.28	0.29	5.65
	>=1.46	119	27.9	12	36.4	1.06	0.41	2.77	117	27.1	14	51.9	3.33	0.90	12.26
Gestational age at inclusion (weeks)	<10	101	23.7	8	24.2	Ref			101	23.4	8	29.6	Ref		
	[10-13]	209	49.1	13	39.4	0.85	0.33	2.18	212	49.1	10	37.0	0.66	0.24	1.77
	>13	116	27.2	12	36.4	1.37	0.52	3.60	119	27.6	9	33.3	1.14	0.40	3.22
Transportation time (days)	1	192	45.3	12	36.4	Ref			198	46.1	6	22.2	Ref		
	2 or 3	142	33.5	12	36.4	1.44	0.62	3.35	143	33.3	11	40.7	2.45	0.87	6.93
	>=4	90	21.2	9	27.3	1.61	0.65	4.04	89	20.7	10	37.0	3.41	1.18	9.83
Storage duration in days (between collection and analysis)	<865	133	31.2	12	36.4	Ref			135	31.3	10	37.0	Ref		
	[865-1096[	136	31.9	9	27.3	0.80	0.32	1.99	137	31.7	8	29.6	0.85	0.31	2.28
	[1096-1376[	132	31.0	12	36.4	1.02	0.43	2.39	137	31.7	7	25.9	0.67	0.24	1.86
	>1376	25	5.9	0	0.0	-			23	5.3	2	7.4	1.18	0.23	6.00

[a]samples with HNO3 stabilizer only

[b] adjusted for urinary creatinine level, gestational age at inclusion, transportation time, storage duration and self-report of exposure to products that may contain solvents

eAppendix 2: Appendix describing the methods of indirect assessment of occupational exposure to solvents

Women included in the analysis of occupational exposure were those who reported having worked between the beginning of their pregnancy and the date of their inclusion (before 19 weeks of gestation). Only the last occupational activity during this period was recorded. Of the 3005 women who reported working at the beginning of pregnancy, only 144 (4.8%) started a job after the pregnancy began.

Solvent exposure at work was defined both from a self-administered questionnaire at inclusion and by use of a JEM combined with occupation and industrial activity. Details of the process have been published previously (Garlantezec et al. 2009). The questionnaire completed by the pregnant woman at inclusion asked about frequency of contact at work with 11 classes of products considered to be the principal solvent-containing products in addition to solvents used directly (as in the chemical industry or laboratories): paints, strippers, dyes or inks, glues, varnishes, wood sealants, detergents and cleaning agents, grease removers, gasoline, textile treatments, and cosmetics. From these reported frequencies, we built an exposure index in three classes: “never”, “occasionally”, or “regularly exposed to products containing solvents”.
The JEM used in our study (Ferrario et al. 1988; Cordier et al. 1997) is a generic one, which assigned a probability of solvent exposure to a combination of two codes [one occupation code (International Standard Classification of Occupations of the International Labour Office 1968) and one code of industrial activity (International Standard Industrial Classification of all Economic Activities of the United Nations 1975)]. This JEM originally classified exposure in 5 different levels: 10. job-related exposure is not higher than for the general population; 20. job may entail a cumulative exposure higher than for the general population; 3x. job may entail exposure to a level definitely higher than for the general population (31. probability <1/3; 32. probability 1/3-2/3; 33. probability >2/3); 40. job entails exposure to the specific agent at a level clearly higher than for the general population; and 50. job entails exposure to the specific agent and exposure is known to be particularly high. To have enough subjects in each category and according to the bimodal distribution of the initial levels, we combined these exposure categories into 3 groups: no exposure (level 10), medium exposure (levels 20 and 31), and high exposure (levels 32, 33, 40 and 50).

Cordier S, Lefeuvre B, Filippini G, et al. Parental occupation, occupational exposure to solvents and polycyclic aromatic hydrocarbons and risk of childhood brain tumors (Italy, France, Spain). Cancer Causes Control 1997; 8:688-697.

Ferrario F, Continenza D, Pisani P, et al. Description of a job-exposure matrix for sixteen agents which are or may be related to respiratory cancer. In: Hogstedt C, Reuterwall C, eds. Progress in occupational Epidemiology. Amsterdam, Netherlands: Elsevier Science Publishers 1988:379-382.

Garlantézec R, Monfort C, Rouget F, Cordier S. Maternal occupational exposure to solvents and congenital malformations: a prospective study in the general population. Occup Environ Med. 2009;66(7):456-463.

eAppendix 3: Tables and Appendix describing the levels of metabolites and the correlations between urinary metabolites, products handled, and indirect assessment of solvent exposure

Table 3.1. Distribution of urinary levels of alkoxycarboxylic acids, TCAA, and TCOH among 580 control mothers

Urinary Metabolites	>=LOD	25th percentile	50th percentile	75th percentile	Max	Median (of values>LOD)
	No. (%)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Alkoxycarboxylic acids (LOD=0.05 mg/L)						
MAA	167 (28.8)	<LOD	<LOD	0.06	2.97	0.09
MEAA	319 (55.0)	<LOD	0.06	0.26	3.90	0.23
EAA	23 (4.0)	<LOD	<LOD	<LOD	0.62	0.07
EEAA	105 (18.1)	<LOD	<LOD	<LOD	30.00	0.09
PAA	62 (10.7)	<LOD	<LOD	<LOD	0.24	0.07
BAA[a]	424 (92.4)	0.08	0.10	0.16	0.62	0.13
PhAA	539 (92.9)	0.14	0.36	1.27	26.80	0.43
2-MPA	30 (5.2)	<LOD	<LOD	<LOD	0.72	0.13
Chlorinated metabolites (LOD=0.01 mg/L)						
TCAA[a]	33 (7.2)	<LOD	<LOD	<LOD	0.70	0.03
TCOH[a]	27 (5.9)	<LOD	<LOD	<LOD	0.40	0.02

Abbreviations: LOD: limit of detection; MAA: methoxyacetic acid; MEAA: methoxy-ethoxyacetic acid; EAA: ethoxyacetic acid; EEAA: ethoxy-ethoxyacetic acid; PAA: n-propoxyacetic acid; BAA: butoxyacetic acid; PhAA: phenoxyacetic acid; 2-MPA: methoxyproprionic acid; TCAA: trichloroacetic acid; TCOH: trichloroethanol.

[a] excluding samples using hydrochloric acid as stabilizer.

Correlations between urinary metabolites, products handled, and indirect measures of exposure to solvents

Correlations between metabolites

Correlations between metabolites were measured with Chi square or Fisher's exact tests, Tobit regression or Spearman correlation rank, depending on the level of detection. We observed several strong associations:

- MAA detection was associated with MEAA level;
- Detection of TCAA and of TCOH were strongly correlated;
- EEAA detection was associated with PhAA level;
- PAA detection was associated with 2-MPA and with BAA level.

Correlations between MAA and MEAA or TCAA and TCOH were expected since they arise from the same metabolic pathways. Several of the precursor compounds of these metabolites are known components of cleaning agents and cosmetics (Catala et al., 1999).

Correlations between urinary metabolites, products handled, and indirect assessment of solvent exposure

In our control sample (n=507 control women who reported working at inclusion), we observed a number of associations between aggregations of metabolites occurring together and use at work of products that could contain solvents (Table 3.2) and with indirect measures of exposure through self-report or the job-exposure matrix (JEM) (Table 3.3).

The associations were studied according to the following rules: the reference group for each compound was the group of women who reported never having been exposed to this specific compound at work. Metabolites identified in more than half the samples were analyzed as continuous variables. Multiple Tobit regression was used with log-transformed metabolite values as the dependent variable censored at the LOD. Beta coefficients are presented with the corresponding p-value for each comparison. Metabolites identified in fewer than half the subjects were defined in two classes (<LOD vs. >=LOD). Odds ratios (OR) and their 95% confidence intervals were used to express the relations between metabolites that were found compared with not found. Logistic regression was used to adjust for potential confounders.

Regular exposure to cleaning agents at work was associated with the presence of EAA, EEAA, BAA, TCAA, and TCOH. Exposure to cosmetics was associated with EEAA and PhAA. MAA was observed less often for women who reported occupational exposure to detergents and cleaning agents. Grease remover exposure was associated with a higher rate of EEAA and exposure to gasoline with EEAA (Table 3.2).
We also studied the relation between indirect assessments of solvent exposure and the presence of metabolites (Table 3.3). EAA, EEAA, BAA, and TCOH were more frequent in urine when self-reported exposure to solvents was reported, whereas MAA showed the opposite trend. Women defined by the global JEM as exposed to solvents had significantly higher detectable levels of BAA and PhAA and a higher rate of TCOH.

References

Catala M, Cordier S, Delaforge M, Fenaux P, Garnier R, Multigner L, Rico-lattes I, Vasseur P. Éthers de glycol. Quels risques pour la santé ? [in French]. Expertise collective Inserm, Inserm, Paris, ISBN 2-85598-761-X, 1999

Garlantezec R, Multigner L, Labat L, Bonvallot N, Pulkkinen J, Dananche B, et al. Urinary biomarkers of exposure to glycol ethers and chlorinated solvents during pregnancy: determinants of exposure and comparison with indirect methods of exposure assessment. Occup Environ Med 2012 Jan;69(1):62-70.

Helsel DR, Hirsch M. Methods for data below the reporting Limit. In : Techniques of Water-Resources Investigations of the United States Geological Survey. Book 4, Hydrologic Analysis and Interpretation. Chapter A3 Statistical Methods. Eds : U.S. Geological Survey. 2002.

Lubin JH, Colt JS, Camann D et al. Epidemiologic evaluation of measurement data in the presence of detection limits. Environ Health Perspect. 2004 Dec;112:1691-6.

Table 3.2: Associations between alkoxycarboxylic acids, TCAA, and TCOH in urine and occupational exposure to products that might contain solvents (n=507 control women who reported working at inclusion)

	Methoxyacetic acid (MAA)				Methoxy-ethoxy acetic acid (MEAA)			Ethoxyacetic acid (EAA)				Ethoxyethoxyacetic acid (EEAA)			
	< LOD n	>=LOD n	OR	95%CI	GM[a] (mg/L)	Beta-coef logMEAA[b]	p-value	<LOD n	>=LOD n	OR	95%CI	< LOD n	>=LOD n	OR	95%CI
No exposure to paints	320	120	Ref		0.09	Ref		440	17	Ref		378	88	Ref	
Occasional or regular exposure to paints	26	7	0.63	0.3-1.5	0.08	-0.14	0.56	30	3	2.59	0.7-9.3	14	6	1.60	0.7-3.6
No exposure to strippers	328	138	Ref		0.09	Ref		448	18	Ref		363	85	Ref	
Occasional or regular exposure to strippers	15	5	0.79	0.3-2.2	0.10	-0.37	0.20	18	2	2.77	0.6-12.8	28	10	1.84	0.7-4.9
No exposure to dyes or inks	317	131	Ref		0.09	Ref		430	18	Ref		281	53	Ref	
Occasional or regular exposure to dyes or inks	26	12	1.12	0.5-2.3	0.10	0.11	0.62	36	2	1.33	0.3-5.9	20	11	1.53	0.7-3.3
No exposure to glues	298	125	Ref		0.09	Ref		407	16	Ref		339	84	Ref	
Occasional or regular exposure to glues	47	19	0.96	0.5-1.7	0.09	-0.01	0.98	62	4	1.64	0.5-5.1	54	12	0.90	0.5-1.8
No exposure to detergents and cleaning agents	220	110	Ref		0.09	Ref		321	9	Ref		274	56	Ref	
Occasional exposure to detergents and cleaning agents	49	15	0.61	0.3-1.1	0.08	-0.14	0.41	62	2	1.15	0.2-5.5	51	13	1.24	0.6-2.4
Regular exposure to	80	22	0.55	0.3-0.9	0.09	0.03	0.81	92	10	3.88	1.5-9.8	73	29	1.94	1.2-3.3

detergents and cleaning agents			p trend=0.01		p trend=0.98					p trend=0.01				p trend=0.01
No exposure to grease removers	314	133	Ref		0.09	Ref		428	19	Ref		363	84	Ref	
Occasional or regular exposure to grease removers	28	9	0.76	0.3-1.6	0.07	-0.24	0.28	37	0	-	-	26	11	1.83	0.9-3.8
No exposure to gasoline	330	136	Ref		0.09	Ref		447	19	Ref		377	89	Ref	
Occasional or regular exposure to gasoline	16	7	1.06	0.4-2.6	0.11	-0.19	0.49	23	0	-	-	15	8	2.26	0.9-5.5
No exposure to cosmetics	329	131	Ref		0.09	Ref		443	17	Ref		378	82	Ref	
Occasional or regular exposure to cosmetics	17	12	1.77	0.8-3.8	0.12	-0.04	0.89	26	3	3.00	0.8-10.9	15	14	4.30	2.0-9.3

[a] GM: geometric means [b] adjusted for gestational age at inclusion, creatinine level, BMI, storage duration, and alcohol consumption

Table 3.2: (continued): Associations between alkoxycarboxylic acids, TCAA, and TCOH in urine and occupational exposure to products that might contain solvents (n=507 control women who reported working at inclusion)

	2-Butoxyacetic acid (BAA)			n-Propoxyacetic acid (PAA)				Phenoxyacetic acid (PhAA)			Methoxypropionic acid (2-MPA)			
	GM[a] (mg/L)	Beta-coef log BAA[c]	p value	< LOD n	>=LOD n	OR	95% CI	GM[a] (mg/L)	Beta-coef log PhAA[d]	p value	< LOD n	>=LOD n	OR	95%CI
No exposure to paints	0.12	Ref		403	54	Ref		0.41	Ref		431	26	Ref	
Occasional or regular exposure to paints	0.10	-0.13	0.26	33	0	-		0.39	0.07	0.78	32	1	0.52	0.1-3.9
No exposure to strippers	0.12	Ref		415	51	Ref		0.40	Ref		439	27	Ref	
Occasional or regular exposure to strippers	0.10	-0.15	0.32	17	3	1.47	0.4-5.1	0.37	-0.06	0.85	20	0	-	
No exposure to dyes or inks	0.13	Ref		396	52	Ref		0.40	Ref		424	24	Ref	
Occasional or regular exposure to dyes or inks	0.12	-0.01	0.93	36	2	0.42	0.1-1.8	0.52	0.28	0.23	35	3	1.51	0.4-5.3
No exposure to glues	0.12	Ref		372	51	Ref		0.40	Ref		399	24	Ref	
Occasional or regular exposure to glues	0.11	-0.01	0.99	62	4	0.47	0.2-1.3	0.41	0.05	0.79	62	4	1.07	0.4-3.2
No exposure to detergents and cleaning agents	0.12	Ref		296	36	Ref		0.41	Ref		331	19	Ref	
Occasional exposure to detergents and cleaning agents	0.12	0.04	0.63	56	8	1.17	0.5-2.6	0.31	-0.24	0.20	61	3	0.81	0.2-2.8
Regular exposure to	0.13	0.21	0.009	91	11	0.99	0.5-2.1	0.46	0.22	0.17	97	5	0.84	0.3-2.3

detergents and cleaning agents		p trend=0.01				p trend=0.96			p trend=0.33				p trend =0.70	
No exposure to grease removers	0.12	Ref		396	51	Ref		0.40	Ref		422	25	Ref	
Occasional or regular exposure to grease removers	0.11	-0.04	0.73	35	2	0.44	0.1-1.9	0.59	0.16	0.48	36	1	0.47	0.1-3.6
No exposure to gasoline	0.12	Ref		414	52	Ref		0.40	Ref		441	25	Ref	
Occasional or regular exposure to gasoline	0.11	-0.11	0.41	20	3	1.19	0.3-4.2	0.38	-0.09	0.75	21	2	1.68	0.4-7.6
No exposure to cosmetics	0.12	Ref		411	49	Ref		0.39	Ref		333	27	Ref	
Occasional or regular exposure to cosmetics	0.14	-0.09	0.49	24	5	1.75	0.6-4.8	0.77	0.49	0.06	29	0	-	

[a] GM: geometric means [c]adjusted for storage duration [d] adjusted for creatinine and storage duration

Table 3.2: (continued): Associations between alkoxycarboxylic acids, TCAA, and TCOH in urine and occupational exposure to products that might contain solvents (n=507 control women who reported working at inclusion)

	Trichloroacetic acid (TCAA)				Trichloroethanol (TCOH)			
	< LOD n	>=LOD N	OR	95%CI	< LOD n	>=LOD n	OR[e]	95%CI
No exposure to paints	334	25	Ref		340	19	Ref	
Occasional or regular exposure to paints	25	2	1.07	0.2-4.8	23	4	2.48	0.7-9.1
No exposure to strippers	341	26	Ref		346	21	Ref	
Occasional or regular exposure to strippers	16	0	-		15	1	1.10	0.1-8.7
No exposure to dyes or inks	330	24	Ref		334	20	Ref	
Occasional or regular exposure to dyes or inks	26	2	1.06	0.2-4.7	26	2	0.70	0.1-5.4
No exposure to glues	312	24	Ref		316	20	Ref	
Occasional or regular exposure to glues	46	3	0.85	0.2-2.9	46	3	0.74	0.2-3.3
No exposure to detergents and cleaning agents	245	14	Ref		247	12	Ref	
Occasional exposure to detergents and cleaning agents	48	3	1.10	0.3-4.0	50	1	0.41	0.1-3.3
Regular exposure to detergents and cleaning agents	67	10	2.61 p trend=0.03	1.1-6.1	67	10	3.34 p trend=0.02	1.4-8.2
No exposure to grease removers	330	26	Ref		336	20	Ref	
Occasional or regular exposure to grease removers	23	1	0.55	0.1-4.3	21	3	1.80	0.4-8.3
No exposure to gasoline	339	26	Ref		346	19	Ref	
Occasional or regular exposure to gasoline	20	0	-		16	4	3.84	1.0-14.5
No exposure to cosmetics	337	24	Ref		341	20	Ref	
Occasional or regular exposure to cosmetics	22	2	1.28	0.3-5.8	22	2	1.77	0.4-8.2

[e] adjusted for alcohol consumption

Table 3.3: Associations between alkoxycarboxylic acids, TCAA, and TCOH in urine and indirect assessment of exposure to solvents (n=507 control women who reported working at inclusion)

	Methoxyacetic acid (MAA)			
	< LOD n	>= LOD n	OR	95% CI
Self-reported exposure				
Never	179	97	Ref	
Occasional	61	21	0.64	0.4-1.1
Regular	107	32	0.55	0.3-0.9
				p trend =0.008
JEM-assessed exposure				
No	279	120	Ref	
Yes	75	26	0.81	0.5-1.3
	Methoxy-ethoxyacetic acid (MEAA)			
	GM[a] (mg/L)		b coef log MEAA[b]	p value
Self-reported exposure				
Never	0.09		Ref	
Occasional	0.08		- 0.10	0.51
Regular	0.09		-0.05	0.70
				p trend =0.64
JEM-assessed exposure				
No	0.09		Ref	
Yes	0.08		-0.11	0.46
	Ethoxyacetic acid (EAA)			
	< LOD n	>= LOD n	OR	95% CI
Self-reported exposure				
Never	270	6	Ref	
Occasional	81	1	0.56	0.1-4.7
Regular	125	14	5.04	1.9-13.4
				p trend=0.001
JEM-assessed exposure				
No	384	15	Ref	
Yes	95	6	1.71	0.6-4.6
	Ethoxy-ethoxyacetic acid (EEAA)			
	< LOD n	>= LOD n	OR	95% CI
Self-reported exposure				
Never	228	48	Ref	
Occasional	67	15	1.06	0.6-2.0
Regular	104	35	1.60	1.0-2.6
				p trend =0.07
JEM-assessed exposure				
No	326	73	Ref	
Yes	77	24	1.39	0.8-2.4

[a] GM: geometric means [b] adjusted for gestational age at inclusion, creatinine level, BMI, storage duration, and alcohol consumption

[c] adjusted for duration of storage

Table 3.3 (continued): Associations between alkoxycarboxylic acids, TCAA, and TCOH in urine and indirect assessment of exposure to solvents (n=507 control women who reported working at inclusion)

	2-Butoxyacetic acid (BAA)			
	GM[a] (mg/L)		B coef log BAA[c]	p value
Self-reported exposure				
Never	0.11		Ref	
Occasional	0.11		0.01	0.92
Regular	0.13		0.21	0.005
				p trend=0.008
JEM-assessed exposure				
No	0.11		Ref	
Yes	0.14		0.27	0.0006
	n-Propoxyacetic acid (PAA)			
	< LOD N	>= LOD n	OR	95% CI
Self-reported exposure				
Never	245	31	Ref	
Occasional	75	7	0.74	0.3-1.7
Regular	121	18	1.17	0.6-2.2
				p trend=0.69
JEM-assessed exposure				
No	353	46	Ref	
Yes	93	8	0.66	0.3-1.5
	Phenoxyacetic acid (PhAA)			
	GM[a] mg/L		B coef log PhAA[d]	p-value
Self-reported exposure				
Never	0.40		Ref	
Occasional	0.34		-0.10	0.55
Regular	0.46		0.14	0.33
				p trend=0.40
JEM-assessed exposure				
No	0.38		Ref	
Yes	0.53		0.26	0.09
	2-Methoxypropionic acid (2-MPA)			
	< LOD N	>= LOD n	OR	95% CI
Self-reported exposure				
Never	258	18	Ref	
Occasional	78	4	0.74	0.2-2.2
Regular	133	6	0.65	0.3-1.7
				p trend=0.34
JEM-assessed exposure				
No	374	25	Ref	
Yes	98	3	0.46	0.1-1.5

[a] GM: geometric means [c]adjusted for duration of storage [d] duration of storage and creatinine level

Table 3.3 (continued): Associations between alkoxycarboxylic acids, TCAA, and TCOH in urine and indirect assessment of exposure to solvents (n=507 control women who reported working at inclusion)

	Trichloroacetic acid (TCAA)			
	< LOD n	>= LOD n	OR	95% CI
Self-reported exposure				
Never	203	14	Ref	
Occasional	63	4	0.92	0.3-3.0
Regular	93	10	1.56	0.7-3.6
				p trend=0.33
JEM-assessed exposure				
No	300	21	Ref	
Yes	61	8	1.88	0.8-4.4
	Trichloroethanol (TCOH)			
	< LOD n	>= LOD n	OR[e]	95% CI
Self-reported exposure				
Never	207	10	Ref	
Occasional	65	2	0.36	0.0-2.9
Regular	91	12	3.01	1.2-7.4
				p trend=0.03
JEM-assessed exposure				
No	305	16	Ref	
Yes	61	8	2.90	1.2-7.2

[e] adjusted for alcohol consumption