ORIGINAL ARTICLE

The Upper Midwest Health Study: gliomas and occupational exposure to chlorinated solvents

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ABSTRACT

Objectives Occupational exposure to chlorinated aliphatic solvents has been associated with an increased cancer risk, including brain cancer. However, many of these solvents remain in active, large-volume use. We evaluated glioma risk from non-farm occupational exposure (ever/never and estimated cumulative exposure) to any of the six chlorinated solvents—carbon tetrachloride, chloroform, methylene chloride, trichloroethylene, tetrachloroethylene or 1,1,1-trichloroethane—among 798 cases and 1175 population-based controls, aged 18–80 years and non-metropolitan residents of Iowa, Michigan, Minnesota and Wisconsin. Methods Solvent use was estimated based on occupation, industry and era, using a bibliographic database of published exposure levels and exposure determinants. Unconditional logistic regression was used to calculate ORs adjusted for frequency matching variables age group and sex, and age and education. Additional analyses were limited to 904 participants who donated blood specimens (excluding controls reporting a previous diagnosis of cancer) genotyped for glutathione-*S*-transferases *GSTP1, GSTM3* and *GSTT1*. Individuals with functional *GST* genes might convert chlorinated solvents crossing the blood–brain barrier into cytotoxic metabolites.

Results Both estimated cumulative exposure (ppm-years) and ever exposure to chlorinated solvents were associated with decreased glioma risk and were statistically significant overall and for women. In analyses comparing participants with a high probability of exposure with the unexposed, no associations were statistically significant. Solvent-exposed participants with functional *GST* genes were not at increased risk of glioma.

Conclusions We observed no associations of glioma risk and chlorinated solvent exposure. Large pooled studies are needed to explore the interaction of genetic pathways and environmental and occupational exposures in glioma aetiology.

What this study adds

- Chlorinated solvents are known neurotoxicants.
- Chlorinated solvents are probable carcinogens.
- Quantitative chlorinated solvent exposure is not associated with an increased risk of glioma.
- Glutathione-S-transferase genotypes do not affect the glioma-solvent association among those exposed to chlorinated solvents.

INTRODUCTION

Six chlorinated solvents—carbon tetrachloride, chloroform, methylene chloride, trichloroethylene, tetrachloroethylene and 1,1,1-trichloroethane—have been in wide commercial use with millions of workers worldwide exposed.[1] The International Agency for Research on Cancer (IARC) has evaluated carbon tetrachloride, chloroform and methylene chloride as possible human carcinogens, trichloroethylene and tetrachloroethylene as probable human carcinogens and 1,1,1-trichloroethane as having insufficient data to evaluate human carcinogenicity.[2–6] IARC has scheduled a meeting for October 2012 to re-evaluate the assessments of trichloroethylene and tetrachloroethylene.

Much has been written on the health effects, especially cancer, associated with exposure to these solvents, including a number of recent reviews[1 7–10] but there are few studies of chlorinated solvent exposure and brain cancer, and fewer limited to gliomas. A case-only study in Shanghai, China, assigned women with brain cancer a low or high level of exposure to organic solvents, based on occupation. Those with a high probability of high solvent exposure had a nearly two-fold risk.[11] A case–control study in Sweden found a greater than two-fold relative risk of glioma for men who self-reported exposure to 'solvents, degreasers or cleaning agents'.[12] There was no significant increase in risk for women. Three consecutive case–control studies of glioma and other cause deaths used occupational information from death certificates,[13] next-of-kin interviews[14] and job-exposure matrices[15 16] to estimate solvent exposure with the strongest association for methylene chloride and risk of glioma with increasing probability of exposure and with increasing duration of exposure in high-exposed jobs.[15] Using a different set of job-exposure matrices associating women's occupations on death certificates with estimated intensity and probability of exposure to chlorinated solvents, Cocco *et al*[17] found an increased risk for solvents and, in particular, for methylene chloride by increasing probability of exposure, but not by intensity of exposure.

The glutathione-*S*-transferase (GST) enzymes are involved in phase II detoxification pathway for chlorinated solvents (the phase I pathway employs cytochrome P450).[18] Although GST is considered a detoxification pathway for many chemicals, in the case of certain chlorinated solvents, it is the GST pathway that has been most strongly implicated in genotoxicity and carcinogenicity.[19 20] Humans with fully functional *GST* genes (*GST*-positive) could theoretically produce enzymes that metabolise chlorinated solvents to cytotoxic metabolites; those with less functional or nonfunctioning genes (*GST*-null) have little or no enzyme and apparently do not produce cytotoxic metabolites from chlorinated solvent exposure. GSTP1 and GSTM3 enzymes are produced in the brain[21–23] and GSTT1 is expressed in the brain.[24] Some studies have found an association between *GSTP1* genotypes and glioma risk[19 25 26] although other studies have seen no increased risk.[20 21 23] It has also been reported that *GSTT1*-null individuals are at increased risk of glioma.[27] Because chlorinated solvents can cross the blood–brain barrier[28] the cytotoxic metabolites might be produced in brain tissue in individuals with fully functional GSTP1, GSTM3 or GSTT1 enzymes.

In 1995, the National Institute for Occupational Safety and Health (NIOSH) initiated the Upper Midwest Health Study (UMHS), a population-based case–control study of glioma risk in a non-metropolitan population. The main focus was farming and associated rural risk factors.[29] The questionnaire also included a complete non-farm occupational history so 'exposures of interest' on non-farming jobs, including those to chlorinated solvents, could be assessed.

The analyses presented here evaluated associations between risk of glioma and exposures from non-farming jobs (ever/never and estimated cumulative) to any of six chlorinated solvents—carbon tetrachloride, chloroform, methylene chloride, trichloroethylene, tetrachloroethylene or 1,1,1-trichloroethane—among study participants. We did not consider farm jobs when evaluating chlorinated solvent exposure because the literature on farmer solvent use was very meagre and because farmers tend to use non-chlorinated solvents for farm tasks, as our paper on farm activities reported.[30] However, all non-farm jobs reported by farmers were evaluated for chlorinated solvent exposure. The primary hypothesis was that at least one of these chlorinated solvents would be associated with increased glioma risk. In addition, we hypothesised that among genotyped participants risk would differ by genotype.

MATERIALS AND METHODS

The study sample and design have been described previously.[29] Residents of non-metropolitan counties of Iowa, Michigan, Minnesota and Wisconsin at diagnosis (cases) between 1 January 1995 and 31 January 1997 or resident (controls) on 1 January 1995 were eligible to participate. The four study states have large farm populations and higher than average brain cancer incidence. Using the distribution by sex and age at diagnosis (by ten-year age groups) of gliomas during a three-year period (1989–1992) in the study states, we selected potential controls (2:1 to projected number of cases) from state driver's license records (ages 18 to 64 years) or from Health Care Financing Administration's (HCFA) Medicare data tapes (ages 65–80 years). Sampling randomly within sex-age strata across eligible counties, we chose a pool of potential controls as the case enrolment began. Participants reporting prior malignancies other than glioma (6.4% of cases, 20.6% of controls) were not excluded.

The study focused on histologically confirmed primary intracranial gliomas, (ICD-O code 938–948),[31] rather than all brain neoplasms, to reduce heterogeneity among the case participants. Cases were identified through participating medical facilities and neurosurgeon offices. Case ascertainment, assessed by comparison with respective state tumour registries for eligible counties, was 78% overall.[29] Physician consent was obtained before contacting cases or their next-of-kin. Potential participants received a letter of invitation and a follow up telephone call to request participation. Informed consent was solicited from all potential participants. Among eligible potential participants, 70.4% of 1669 controls and 91.5% of 872 cases (or their next-of-kin) agreed to participate, resulting in a study population of 1175 controls and 798 cases.[29] Interviews with participants (many with family members also taking part) were conducted for 199/462 (43%) glioblastoma cases, 239/336 (72%) other glioma cases and 1141/1175 (97%) controls. Cases (n=438) were interviewed in person at an average of 196 days after diagnosis; proxy case interviews (n=360) occurred at an average of 420 days after diagnosis (partly due to waiting after a case death before approaching family members). Among cases, 58% had a diagnosis of glioblastoma multiforme (equivalent to stage 4 glioma); 22%, astrocytoma; 11%, oligodendroglioma; 8%, other glioma subtypes.

This study was approved by the NIOSH Human Subjects Review Board (HSRB 94-DSHEFS-08) and review boards of all participating institutions.

The questionnaire, modified from one developed by the National Cancer Institute,[32] included a complete occupational history. Respondents were asked about all jobs of at least a year's duration between the age of 16 years and the end of 1992, including employer name, industry, job title, job tasks, materials used and employment frequency. The questionnaire also asked specifically about certain exposures, including 'solvents, thinners, glues, inks, varnishes, stains or paint strippers', and on which jobs and for how many hours a week these exposures occurred.

For this analysis, the probability, intensity and frequency of exposure to six chlorinated solvents—carbon tetrachloride, chloroform, methylene chloride, trichloroethylene, tetrachloroethylene and 1,1,1-trichloroethane—in non-farm jobs was estimated based on occupation, industry and decade, using our annotated appendix of sources of exposure data[33] as well as bibliographic databases of published exposure levels.[34 35] We estimated values for a set of exposure determinants,[33] with an algorithm linking participants, jobs and exposure determinants (The process is illustrated graphically in the online supplementary figure S1).

The jobs dataset contained 12 145 observations (participant-jobs) for 1967 participants (six participants were 16 years of age in 1993 with no jobs recorded), including 4067 observations of gaps in the work histories. Information reported by the respondent on employment frequency was used to assign two quantitative factors: employment status (full or part time) and seasonality, based on the fraction worked during a year. In the absence of information, these were assumed to be 1. This information was necessary for cumulative exposure calculations (described below).

The exposure file contained six records for each job, one for each solvent. An industrial hygienist (IH), blinded to case–control status, reviewed the job information and assigned the following for each of the chlorinated solvents: (1) Probability of exposure (0=not exposed, 1=<0.1, 2=0.1–0.49, 3=0.50–0.89, 4>0.89), (2) frequency of exposure (0: not exposed,

1:1 h/week, 2: 2–10 h/week, 3: 11–20 h/week, 4: >20 h/week), (3) degree of confidence in the probability assignment (1=no information, 2=solvent present, 3=solvent use described relatively, 4=solvent use described quantitatively) and (4) degree of confidence in the frequency assignment (1=low to 4=high).

For jobs with probability of exposure >0, the IH additionally assigned values for the following exposure determinants: (1) primary mechanism of release, (2) secondary mechanism of release, (3) ventilation type and effectiveness, (4) process condition, (5) quantity, (6) temperature, (7) proximity to source, (8) location (indoor/outdoor), (9) confined space and (10) an indication of the confidence associated with the determinant assignments. These determinants were used to model intensity from measurement data in the published literature. Details about the exposure determinants and how they were used in computing exposure levels have been described.[33]

Exposure status was assigned to each job. Jobs assigned a zero probability of exposure were considered to be not exposed and assigned 0 ppm-years for continuous cumulative exposure variables. Jobs assigned a probability of exposure >0 were considered to be exposed and a continuous cumulative exposure was estimated as follows. First, each job was split so as to have one observation for each calendar year the job was held and duration of exposure (days) for each year the job was held, was calculated from the split job start and end dates. Next, exposure frequency factor values were assigned as 1 for '1 h/week', 6 for '2–10 h/week', 15 for '11–20 h/week', and 30 for '>20 h/week'. These were converted to 1/7 (0.14) h/day, 6/7 (0.86) h/day, 15/7 (2.14) h/day, or 30/7 (4.30) h/day for cumulative exposure calculations. For methylene chloride, 1,1,1-trichloroethane and trichloroethylene, exposure intensity (ppm) was calculated from 1975 (1955 for trichloroethylene) to 1995 using the exposure-determinants models as previously described.[33] The modelled intensity for 1975 was assigned to all prior years for methylene chloride and 1,1,1-trichloroethane whereas the modelled intensity for 1955 was assigned to all prior years prior for trichloroethylene, based on the availability of measurements. For carbon tetrachloride, chloroform and tetrachloroethylene, exposure intensity was assigned using the estimated methylene chloride exposure intensity and a vapour pressure conversion factor based on the Ideal Gas Law to convert the estimated methylene chloride exposure intensity to an intensity for each of the other solvents (tetrachloroethylene, carbon tetrachloride and chloroform). Finally, cumulative exposure (ppm-h) for each solvent-worker-job-year was the product of duration (days), employment frequency (unitless), exposure frequency (h/day) and exposure intensity (ppm). Cumulative exposure (ppm-h) for each worker was the sum of the cumulative exposures for all job-years. Exposures were converted to ppm-years, with 1 ppm-year equal to 2000 ppm-h.

Analyses were conducted using dichotomous exposures (ever/never exposed) for each solvent for all participants and separately for men and women. Since exposure to chlorinated solvents has been associated with a number of cancers, we also performed some analyses excluding controls who had reported having had cancer. Unconditional logistic regression modelling adjusted for the frequency-matching variables (10-year age group and sex), and for age and education (less than high school, high school graduate (referent group), post high-school education), as a surrogate for socioeconomic status, to obtain maximum-likelihood parameter estimates for each solvent exposure. Age was included as well as age group to adjust for residual confounding within age groups.[36 37] We repeated the categorical analysis limiting the "exposed" category to participants with a high probability of having been exposed (ie, 0.5 or higher).

Additional analyses used a natural log transformation of continuous exposures in ppm-years, with a small number (−10) substituting for zero exposures. Analyses were repeated separately, for male and female participants and excluding participants with zero estimated exposure. We report results both including and excluding proxy responses in tables (in the text, unless noted, we report only results including proxy responses). For all analyses, we used the SAS V.9.1 software.

Since genetics affects how chemicals are metabolised we wanted to investigate the possible effect of differences in enzymes that metabolise chlorinated solvents, the glutathione-S-transferases. Individuals with functional (positive) *GST* genes might convert chlorinated solvents crossing the blood–brain barrier into cytotoxic metabolites. These analyses excluded 124 cases and 232 control participants who declined to donate blood specimens, 347 cases who were deceased or too ill to ask, 360 controls who were not asked to donate a blood specimen, and, for each analysis, participants whose specimens had not been successfully genotyped. Since exposure to chlorinated solvents has been associated with a number of cancers, these analyses also excluded controls who reported having had cancer (n=111 for *GSTP1* and 120 for *GSTM3* and *GSTT1*).

In analyses restricted to participants genotyped for *GSTP1* (322 cases and 456 controls genotyped), *GSTM3* (316 cases and 443 controls genotyped) and *GSTT1* (319 cases and 450 controls genotyped) we compared risk of glioma by genotype within solvent exposure groups (exposed or unexposed), postulating that any genetic effect would be more pronounced in the solvent-exposed group. In these analyses we do not present results excluding proxies, since all but ten blood donors personally completed the questionnaire interview.

Laboratory methods: Some 325 cases (41% of all participating cases or 71% of 458 alive at the time of interview) and 579 controls (73% of 793 asked) provided blood specimens. DNA was extracted from whole-blood specimens using a sodium perchlorate–chloroform method.[38] At the time of DNA extraction, PCR/restriction fragment length polymorphism reactions were conducted by GenoType, Ltd (UK) to characterise *GSTP1* Il05V rs1695 A/G and A114V rs1138273 C/T using a minor modification of the procedure described by Watson *et al*[39] and GSTT1 (null) was characterised using genotyping methods described by Pemble *et al*[40] and Chenevix-Trench *et al*[41] For *GSTP1*, among 904 specimens, we excluded 9 specimens that lacked genotyping results and 120 specimens from controls who had reported having had cancer, leaving 778 specimens in the analysis.

For *GSTT1*, there were 904 specimens; 15 lacking genotyping results and 120 from controls who had reported having had cancer were excluded, leaving 769 specimens in the analysis.

GSTM3 *A/*B was genotyped at the NCI Core Genotyping Facility, Gaithersburg, MD, using high-throughput (TaqMan) procedures. *B has a 3 base pair deletion in intron 6. Specific details on primers, reaction conditions and amplification procedures are described in http://variantgps.nci.nih.gov/cgfseq/pages/resultSubmit.do?method=getPlatform&assayLid=001_0777.
Among 881 specimens available, 11 lacked genotyping results and 111 were from controls who had reported having had cancer, and were therefore excluded, leaving 759 specimens in the analysis.

RESULTS

Numbers of cases and controls and basic demographics are presented in table 1. Almost all participants had held non-farm

Table 1 Characteristics of cases and controls and risk of glioma, according to respondent status

	Including proxy-only interviews*						Excluding proxy-only interviews					
	Cases		Controls				Cases		Controls			
Characteristic	No.	%	No.	%	OR†	95% CI	No.	%	No.	%	OR†	95% CI
All participants	798		1175				438		1141			
Men	457	57	648	55	1.09	0.91 to 1.31	242	55	625	55	1.02	0.82 to 1.27
Women	341	43	527	45	Referent		196	45	516	45	Referent	
Age in 1993 (mean (SD))	52.3 (16.1)		55.1 (15.4)		0.99	0.98 to 0.99	45.9 (15.3)		54.7 (15.4)		0.97	0.96 to 0.97
Ever had non-farm job (≥1 year)	762	95	1105	94	1.38	0.90 to 2.10	414	95	1076	94	1.07	0.64 to 1.80
Longest job												
Professional	234	31	345	31	Referent		141	34	337	31	Referent	
Trades	296	39	412	37	0.97	0.74 to 1.27	146	35	398	37	0.85	0.61 to 1.18
Service	230	30	346	31	0.94	0.72 to 1.22	125	30	339	32	0.81	0.58 to 1.12
White non-Latino	783	98	1152	98	1.24	0.64 to 2.42	429	98	1119	98	1.33	0.60 to 2.97
Education												
College graduate	132	17	200	17	Referent		89	20	198	17	Referent	
High school graduate	523	66	768	65	1.09	0.80 to 1.40	303	69	752	66	0.98	0.73 to 1.31
<12 years	143	18	207	18	1.27	0.91 to 1.75	46	11	191	17	0.97	0.63 to 1.51
Total	798		1175				438		1141			

*Includes subject+proxy interviews for 137 cases and 49 controls.
†Adjusted for frequency matching variables (age group, sex) and age and education (except for education, which was adjusted for frequency matching variables and age, and age and gender, which were unadjusted).
Modified from table 4 from ref. [25].

jobs, and the distributions of longest jobs over broad categories (professional, trade or service) were similar. When proxy-only interviews were excluded, cases were more likely to have graduated from college and high school than were controls. As reported by us previously, controls were significantly older than cases (table 1)[29]

For 283 cases (35%, 69 women, 214 men) and 475 controls (40%, 144 women, 331 men) occupational exposure to 'solvents, thinners, glues, inks, varnishes, stains or paint strippers' was reported. Based on all the reported occupational information, exposure to at least one solvent was assigned to 359 cases (45%, 244 men, 115 women) and 570 controls (49%, 364 men, 206 women). Ever exposure to each of the six solvents, or to any chlorinated solvent, was associated with a decreased risk of glioma (table 2). Results were similar for men and women considered separately although the odds were higher for men and the CIs for men all included one. When data from proxy interviews were excluded, the odds of a positive association with glioma were slightly higher for men, but still below 1.0. The association did not change when controls who reported having had cancer were excluded (results not shown).

When we repeated the categorical analysis comparing "exposed" participants with a high probability of having been exposed (ie, 0.5 or higher) to the unexposed, results (not shown) were similar. However, because the numbers of "highly probable to have been exposed' participants were much smaller, none of the associations were statistically significant.

Adjusted ORs for estimated cumulative exposure to each of the six solvents were associated with statistically significant reduced risks of glioma (table 3A). In separate analyses for men and women, all ORs were associated with reduced glioma risk but only the results for women were statistically significant (results not shown). We repeated the analysis excluding the unexposed participants (table 3B). The mean cumulative levels were higher than those for all participants, ranging from 3.5 to 98.9 ppm-years, but the odds did not change appreciably; because the numbers of included participants were lower, the CIs were wider and none of the ORs were statistically significant.

Table 4 compares glioma risk separately for chlorinated-solvent-exposed and unexposed cases and controls genotyped for glutathione-*S*-transferases. Proxy-excluded results are not presented because proxy interviews were done for only 10 blood donors. For *GSTM3*, among 881 specimens, 11 lacking genotyping results and 111 from controls who had reported having had cancer were excluded, leaving 759 in the analysis. For *GSTP1*, among 904 specimens, 9 lacking genotyping results and 120 from controls who had reported having had cancer were excluded, leaving 778 in the analysis. For *GSTT1*, among 904 specimens, 15 lacking genotyping results and 120 from controls who had reported having had cancer were excluded, leaving 769 specimens in the analysis.

Participants who did not have the *GSTM3* *B deletion on either chromosome (*GSTM3* *A*A), and therefore had a fully functioning enzyme, could theoretically, if exposed to chlorinated solvents, produce cytotoxic metabolites. However, the association with glioma risk was lower among those exposed to chlorinated solvents than among those not exposed to chlorinated solvents. Participants with the *GSTP1* I105V I->V polymorphism were at a greater risk of glioma in an analysis unadjusted for solvent exposure or other factors (unadjusted OR 1.2, 95% CI 0.9 to 1.6) so we assumed they comprised the higher risk group. *GSTT1*-null participants were at a slightly elevated but not statistically significant risk of glioma. Table 4 shows there was no difference in risk by genotype within or across solvent exposure groups.

DISCUSSION

Occupational exposure to chlorinated solvents has been associated with a number of adverse health effects, including cancer.[1] The association with brain cancer in general, and glioma in particular, has been inconsistent. In our study of exposure to six chlorinated solvents and glioma, we did not find a higher risk of glioma among solvent-exposed

Table 2 Occupational non-farm chlorinated solvent exposure and glioma by gender, according to respondent status

	Including proxy-only interviews						Excluding proxy-only interviews					
	Cases		Controls				Cases		Controls			
Chlorinated solvent	No.	%	No.	%	OR*	95% CI	No.	%	No.	%	OR*	95% CI
Carbon tetrachloride												
All	263	33	442	38	0.79	0.65 to 0.97	141	32	428	38	0.82	0.64 to 1.06
Men	193	42	302	47	0.85	0.66 to 1.08	107	44	291	47	0.95	0.69 to 1.31
Women	70	21	140	27	0.72	0.52 to 1.01	34	17	137	27	0.64	0.41 to 0.99
Chloroform												
All	275	34	458	39	0.77	0.64 to 0.94	153	35	446	39	0.79	0.62 to 1.01
Men	199	44	307	47	0.86	0.67 to 1.09	110	45	298	48	0.91	0.67 to 1.25
Women	76	22	151	29	0.65	0.46 to 0.90	43	22	148	29	0.62	0.41 to 0.93
Methylene chloride												
All	304	38	490	42	0.80	0.66 to 0.97	169	39	475	42	0.81	0.63 to 1.03
Men	222	49	332	51	0.88	0.69 to 1.13	121	50	320	51	0.90	0.66 to 1.23
Women	82	24	158	30	0.69	0.50 to 0.95	48	24	155	30	0.69	0.46 to 1.03
Tetrachloroethylene												
All	299	37	500	43	0.75	0.62 to 0.91	166	38	483	42	0.78	0.61 to 0.99
Men	216	47	338	52	0.81	0.64 to 1.04	117	48	325	52	0.85	0.62 to 1.17
Women	83	24	162	31	0.66	0.48 to 0.91	49	25	158	31	0.68	0.46 to 1.00
Trichloroethane												
All	304	38	503	43	0.75	0.61 to 0.90	173	39	491	43	0.74	0.58 to 0.94
Men	214	47	330	51	0.83	0.64 to 1.06	118	49	321	51	0.83	0.60 to 1.13
Women	90	26	173	33	0.64	0.47 to 0.88	55	28	170	33	0.63	0.43 to 0.93
Trichloroethylene												
All	302	38	515	44	0.74	0.61 to 0.90	164	37	499	44	0.75	0.59 to 0.96
Men	221	48	335	52	0.88	0.69 to 1.12	122	50	323	52	0.97	0.71 to 1.32
Women	81	24	180	34	0.57	0.42 to 0.79	42	21	176	34	0.51	0.34 to 0.77
Any chlorinated solvent												
All	359	45	570	49	0.82	0.68 to 0.99	202	46	553	48	0.86	0.68 to 1.08
Men	244	53	364	56	0.89	0.70 to 1.14	131	54	351	56	0.91	0.66 to 1.25
Women	115	34	206	39	0.73	0.54 to 0.97	71	36	202	39	0.79	0.55 to 1.13

*Adjusted for frequency matching variables (age group, sex) and age and education.

participants. Furthermore, we did not see a difference in risk between men and women. Using a bibliographic database of published exposure levels and exposure determinants, we developed a metric of cumulative chlorinated solvent exposure; however, the findings remained unchanged. Our results suggest that exposure to chlorinated solvents does not increase the risk of glioma.

Study strengths include the large number of histologically confirmed gliomas and the use of population-based controls. Another strength was the estimation of workplace exposure determinants by industrial hygienists blinded to the case–control status of participants, with documented published literature to rigorously estimate intensity. The large percentage of proxy case respondents and possible poor recall by case respondents could have affected the analysis if work details that might be associated with chlorinated solvent exposure were less specific for case than for control responses. Since controls were generally older (table 1) and started working during earlier eras, their opportunity for an assessment of greater exposure by the industrial hygienists was higher. The algorithm for sampling controls

Table 3A Estimated cumulative chlorinated solvent exposure (ppm-years) and risk of glioma, according to respondent status, including unexposed participants

	Including proxy-only interviews								Excluding proxy-only interviews							
	Cases (n=798)			Controls (n=1175)					Cases (n=438)			Controls (n=1141)				
Chlorinated solvents*	Mean	SD	Max	Mean	SD	Max	OR†‡	95% CI	Mean	SD	Max	Mean	SD	Max	OR	95% CI
Carbon tetrachloride	6.0	27.6	373.3	7.3	34.4	784.5	0.98	0.96 to 0.99	5.7	29.5	373.3	7.1	34.1	784.5	0.98	0.96 to 1.00
Chloroform	10.4	45.6	640.5	12.4	58.2	1337.0	0.98	0.96 to 0.99	10.0	47.4	640.5	12.0	57.6	1337.0	0.98	0.96 to 1.00
Methylene chloride	30.1	126.2	1414.0	29.9	129.8	2952.0	0.98	0.97 to 0.99	30.9	143.2	1414.0	29.2	128.7	2952.0	0.98	0.96 to 1.00
Tetrachloroethylene	1.3	5.4	60.8	1.3	5.6	126.9	0.97	0.95 to 0.99	1.3	6.1	60.8	1.3	5.6	126.9	0.97	0.95 to 0.99
Trichloroethane	7.9	34.7	413.7	8.8	40.7	853.5	0.97	0.96 to 0.99	8.3	38.2	413.7	8.7	40.5	853.5	0.97	0.95 to 0.99
Trichloroethylene	32.5	209.2	4046.0	43.3	296.1	5765.0	0.98	0.96 to 0.99	31.5	190.3	2512.0	39.9	284.2	5765.0	0.98	0.96 to 0.99

Table 3B Estimated cumulative chlorinated solvent exposure (ppm-years) and risk of glioma, according to respondent status, excluding unexposed participants

	Including proxy-only interviews								Excluding proxy-only interviews							
	Cases			Controls					Cases			Controls				
Chlorinated solvents*	Mean	SD	N§	Mean	SD	N§	OR†‡	95% CI	Mean	SD	N§	Mean	SD	N§	OR†‡	95% CI
Carbon tetrachloride	18.3	45.7	263	19.5	54.0	442	0.97	0.90 to 1.05	17.7	50.1	141	19.0	53.7	428	0.97	0.88 to 1.07
Chloroform	30.1	73.8	275	31.7	89.9	458	0.98	0.91 to 1.06	28.7	77.0	153	30.8	89.1	446	0.98	0.89 to 1.08
Methylene chloride	78.9	195.0	304	71.8	193.5	490	0.96	0.89 to 1.03	80.0	222.2	169	70.2	192.2	475	0.96	0.87 to 1.05
Tetrachloroethylene	3.5	8.4	299	3.1	8.3	500	0.99	0.93 to 1.07	3.5	9.5	166	3.1	8.2	483	1.01	0.92 to 1.10
Trichloroethane	20.7	53.9	304	20.6	60.2	503	0.97	0.90 to 1.04	21.1	58.6	173	20.3	59.9	491	0.96	0.88 to 1.05
Trichloroethylene	85.9	333.5	302	98.9	441.3	515	1.02	0.95 to 1.10	84.0	304.3	164	91.3	424.5	499	1.05	0.95 to 1.16

*For all six solvents, median and minimum estimated cumulative exposure are 0.
†Adjusted for frequency matching variables (age group, sex) and age and education.
‡OR for a 1-unit increase in natural-log transformed exposures in ppm-years –10 was assigned to log (ppm-years) for those with 0 ppm-years.
§Number of participants estimated to have been exposed, among all participants.

from the motor vehicle registration and Medicare rolls was based on the distribution by gender and age at diagnosis (by ten-year age groups) of glioma cases during a 3-year period (1989–1992) in the four study states. The distribution of diagnosed cases during our study period turned out to be skewed toward younger age at diagnosis; this is how the case–control age difference arose. For this analysis, specific limitations include the lack of detailed information from participants about occupational exposures which could have been used to confirm the exposures estimated on the basis of occupation, industry and decade. Another limitation, from using a statistical model based on published measurements to estimate intensity, is our assumption that the exposure levels in the workplaces of study participants fell within the range of exposures in workplaces reported in the literature.

Most of the earlier studies of solvent exposure and brain cancer had greater limitations. Only one previous study[12] included interviews with cases and controls. In the others, occupational information was obtained entirely from cases,[11] from proxies[13–15] or was based on a single occupation on a death certificate.[17] Of the two population-based participant-interviewed case–control brain cancer studies reporting on solvent exposure to date, Rodvall *et al*[12] reported a positive association based on self-reported and assessed exposure to solvents (benzene, toluene, trichloroethylene and xylene) by 15 cases and 20 controls. With 359 cases and 570 controls with expert-assessed exposure to chlorinated solvents, we found no positive association. Rodvall *et al* study and ours are inconsistent, but had only one solvent (trichloroethylene) in common.

Exposures to chlorinated solvents on the farm were not considered in these analyses. Although all or almost all farmers are exposed to solvents, they generally do not use chlorinated solvents. We reported on solvent use associated with farm tasks; most of the reported use was to gasoline, kerosene or other petroleum-based solvents.[30] Chlorinated solvent use by farmers in non-farm jobs was included in the analyses.

Our findings for the genotyped participants (table 4) were unexpected. Theoretically, if the cytotoxic metabolites of chlorinated solvents were the major cancer risk factor for individuals exposed to chlorinated solvents, we would have expected an increased risk of cancer among participants with the non-deleted genotypes for *GSTP1*, *GSTM3*, and *GSTT1*, who were capable of producing the cytotoxic metabolites, and who were exposed to chlorinated solvents. Our results showed no association. This could be due to the cytotoxic metabolites of chlorinated solvents not being the major risk factor, chlorinated solvent exposure not being a risk factor for glioma, or heterogeneity among the genotypes for other genes in the chlorinated solvent metabolic pathways. Our results are in agreement with a

Table 4 Glioma risk by glutathione *S*-transferase genotypes within occupational nonfarm chlorinated solvent exposure groups

	Solvent exposure						No solvent exposure					
	Cases		Controls				Cases		Controls			
Genotype	No.	%	No.	%	OR†	95% CI	No.	%	No.	%	OR†	95% CI
GSTM3	157/316		241/443				159/316		202/443			
GSTM3 *A*A	111	71	170	71	0.91	0.57 to 1.45	119	75	142	70	1.23	0.74 to 2.05
GSTM3 *A*B or *B*B	46	29	71	29			40	25	60	30		
GSTP1	160/322		245/456				162/322		211/456			
GSTP1- I105V only‡	69	43	98	40	1.21	0.79 to 1.85	68	42	74	35	1.48	0.93 to 2.34
GSTP1-other genotypes§	91	57	147	60			94	58	137	65		
GSTT1	159/319		240/450				160/319		210/450			
GSTT1-	29	18	33	14	1.40	0.78 to 2.50	26	16	28	13	1.10	0.59 to 2.07
GSTT1+	130	82	207	86	0.72	0.40 to 1.29	134	84	182	87	0.91	0.48 to 1.71

†Adjusted for frequency matching variables (age group, sex) and age and education, within solvent exposure groups.
‡One or two copies of the I105V Ile->Val SNP (rs1695) but no copy of the A114V Ala->Val SNP (rs1138272).
§Neither I105V nor A114V variant or with only A114V Ala->Val SNP.

meta-analysis of eight studies (including ours), that did not find an association between GST polymorphisms and the risk of brain tumours.[42]

The associations we saw may be due to some factor, linked to chlorinated solvent exposure that we did not study, such as being fit enough to work in occupations with elevated solvent exposure. A theoretical "unhealthy worker" effect could affect participants with glioma, but only if asymptomatic individuals began to experience problems long before their diagnoses. Recent genetic work provides some support for this theory. Isocitrate dehydrogenase mutations apparently occur frequently in gliomas, mimicking an inborn error of metabolism, L-2-hydroxyglutaric aciduria.[43 44] Individuals born with this neurometabolic disorder have neurological symptoms, fatigue and balance problems, increasing as hydroxyglutamate accumulates.[45] Individuals in whom such mutations occurred later in life might begin experiencing similar symptoms and become less likely to hold jobs requiring moderate or greater physical activity and/or involving machinery, that is, jobs more likely than sedentary desk jobs to be associated with solvent exposure. However, we compared declared 'age at retirement' for those participants who volunteered this information and saw no difference between cases and controls (results not shown). Lack of knowledge about work details by proxies of cases and memory problems in cases, compared with relatively healthy controls; the earlier entry into the work force, during eras in which solvent exposure was higher, of controls; and the slightly higher education level of cases, which could have resulted in their working in jobs with less opportunity for hands-on exposure to solvents, are more likely explanations for the differences we saw in assessed solvent exposure.

CONCLUSION

In our study population, both estimated cumulative exposure (ppm-years) and estimated ever exposure to chlorinated solvents were associated with decreased risk of glioma and were statistically significant overall and for women. Analyses excluding unexposed participants had similar, but not statistically significant, results. In analyses comparing participants with a high probability of exposure with the unexposed none of the associations were statistically significant. Solvent-exposed participants with functional glutathione-*S*-transferases *GSTP1*, *GSTM3* and *GSTT1* were not at increased risk of glioma. Large pooled studies should be undertaken to explore the interaction of genetic pathways and environmental and occupational exposures

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Contributors AMR wrote the manuscript, JHY did the analyses, MAW and PAS conducted the exposure assessment. All authors reviewed the manuscript and contributed ideas to the analysis.

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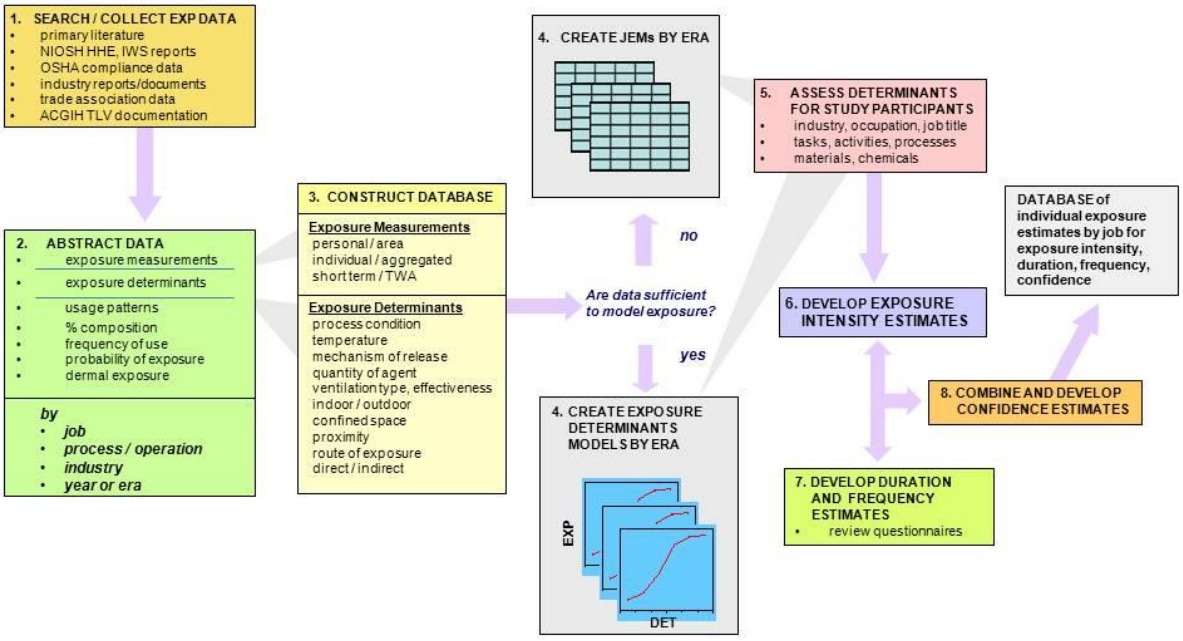

Supplement Figure 1: Exposure Assessment