American Journal of
EPIDEMIOLOGY

Volume 157

Number 6

March 15, 2003

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Bloomberg School of Public Health
Sponsored by the Society for Epidemiologic Research
Published by Oxford University Press

ORIGINAL CONTRIBUTIONS

Maternal Occupational Chemical Exposures and Biotransformation Genotypes as Risk Factors for Selected Congenital Anomalies

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Received for publication April 18, 2002; accepted for publication September 30, 2002.

In a case-control study using an assessment of occupational tasks by an industrial hygienist, the authors investigated whether women's occupational exposures increased risks of delivering infants with cleft palate (CP), cleft lip with or without cleft palate (CLP), conotruncal defects, or limb deficiencies. For CP and CLP, exposures were further considered in the presence/absence of infant genetic variants for glutathione-*S*-transferase M1, glutathione-*S*-transferase T1, and *N*-acetyltransferases 1 and 2. The study included 1987–1989 California stillbirths and livebirths. Telephone interviews were conducted with mothers of 662 CLP and CP cases, 207 conotruncal defect cases, 165 limb deficiency cases, and 734 nonmalformed controls. Occupational tasks were assigned to a priori-defined exposure categories: 74 chemical groups and nine "end-use" chemical groups. Odds ratios of 1.5 or greater were observed for a small number of exposure-defect comparisons. Risks associated with end-use groups revealed odds ratios of 1.5 or greater for exposures to dyes and pigments (conotruncal and CP), propellants (CP), and insecticides (conotruncal and CP). Numerous odds ratios of 2.5 or greater were observed for combined effects of exposures *and* homozygous mutant genotypes, particularly for CP. Although potential associations were observed, most results suggested that maternal occupational chemical exposures did not contribute substantially to the occurrence of these anomalies in this California population.

abnormalities; cleft lip; cleft palate; genes; heart defects, congenital; limb deformities, congenital; occupational exposure; pregnancy

Abbreviations: CI, confidence interval; CLP, cleft lip with or without cleft palate; CP, cleft palate; OR, odds ratio.

Whether occupational exposures early in pregnancy increase risks to women to deliver offspring with congenital anomalies has been the focus of numerous studies (1, 2). Establishing causal effects in these studies is difficult because of several limitations associated with accurate exposure measurements. Most studies, for example, have relied on industry/

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TABLE 1. Frequencies of cases and controls associated with periconceptional maternal occupational exposures* to 74 chemical agent groups, California, 1987–1989

Chemical agent groups	Conotruncal heart cases (no.)	Limb cases (no.)	Controls† (no.)	Isolated CLP‡ cases (no.)	Multiple CLP cases (no.)	Isolated CP‡ cases (no.)	Multiple CP cases (no.)	Controls§ (no.)
Alcohols, aliphatic	27	16	48	39	15	18	9	88
Aldehydes	5	7	19	9	4	3	0	23
Amines, aliphatic	5	0	7	1	2	2	2	10
Amines, aromatic (including aminophenols)	3	0	6	4	0	3	0	12
Ammonia and ammonium hydroxide	11	2	15	14	4	3	6	29
Antibiotics	0	0	1	1	0	0	0	2
Antineoplastic drugs	3	0	0	0	1	0	0	0
Arsenic compounds	1	0	0	0	1	0	0	0
Boron compounds	1	0	2	0	1	0	0	3
Bromides (inorganic)	4	1	3	1	0	1	0	3
Brominated compounds	0	0	0	1	0	0	0	0
Cadmium compounds	0	0	6	0	0	0	0	9
Carbon dioxide	4	3	9	2	1	2	0	11
Carbon monoxide	4	3	9	2	1	2	0	11
Chlorophenoxy herbicides	0	1	1	1	1	0	0	1
Chromium compounds	0	0	3	0	0	0	0	4
Copper compounds	3	0	8	3	0	2	2	15
Dithiocarbamate fungicides/thioureas	0	0	1	0	0	0	0	1
Drugs, not otherwise classified	2	2	3	4	0	2	0	4
Epoxides	1	0	2	0	0	1	0	2
Esters, including formates	4	1	3	3	1	2	0	7
Ethers	2	0	0	2	0	0	0	0
Fluorides	1	1	2	0	1	0	0	2
Formamides and other amides	1	1	0	2	0	0	0	1
Glycol ethers and derivatives	8	2	14	8	3	2	4	25
Glycols	2	0	4	2	0	2	0	6
Halophenols	1	0	0	1	0	1	0	0
Hydrocarbons, aliphatic (C_1–C_4)	4	5	12	9	2	6	2	17
Hydrocarbons, aliphatic (C_5–C_{12})	14	9	18	13	4	8	2	35
Hydrocarbons, aromatic, mononuclear	14	8	21	15	3	8	2	42
Iodine compounds (organic and inorganic)	1	1	1	1	1	2	0	5
Isocyanates	2	0	1	1	0	0	0	1

Table continues

job titles as surrogates for maternal exposures. We recently reported women's risks of having neural tube defect-affected pregnancies from both occupational and nonoccupational chemical exposures using a classification approach that has been successfully used to investigate carcinogenic risks associated with occupational exposures (3–5). Such an approach relies on assessment of occupational tasks by an industrial hygienist and therefore potentially minimizes classification errors associated with proxy exposure measures, such as maternal industry/job titles. The classification process uses a combination of job-exposure linkage and individualized exposure assignment (6), and it is considered a method of choice for assessing occupational risks in case-control studies (7).

In the current study, we examined data from a large California population-based case-control study to investigate whether occupational chemical exposures to women during the periconceptional period increased risks of delivering infants with one of these congenital anomalies: cleft palate, cleft lip with or without cleft palate, conotruncal heart defect, or limb deficiency, phenotypes that have been associated previously with occupational and environmental exposures (1, 2). For a subset of cleft palate and cleft lip with or without cleft palate cases, these potential exposures were further considered in relation to infant genetic polymorphisms for four xenobiotic metabolizing enzymes, glutathione-*S*-transferase M1 (*GSTM1*), glutathione-*S*-trans-

TABLE 1. Continued

Chemical agent groups	Conotruncal heart cases (no.)	Limb cases (no.)	Controls (no.)	Isolated CLP cases (no.)	Multiple CLP cases (no.)	Isolated CP cases (no.)	Multiple CP cases (no.)	Controls (no.)
Ketones	5	3	3	3	1	3	1	8
Lead compounds	0	2	3	2	1	1	0	4
Manganese compounds	0	0	0	0	0	0	0	1
Mercury compounds	1	2	2	1	0	1	1	3
Methacrylates and related compounds	2	0	2	2	0	1	0	5
Nickel compounds	0	0	3	0	0	0	0	4
Nitrates and nitrites	0	0	1	0	0	1	0	1
Nitriles, cyanides, cyanogens	3	0	2	1	0	2	1	5
Nitrogen oxides (except nitrous)	4	3	9	2	2	2	0	11
Nitrous oxide	1	2	6	2	1	1	0	7
Organic acids, derivatives, not otherwise classified	4	1	3	1	2	3	0	4
Organic dyes (excludes aromatic amines)	7	1	3	7	2	0	1	6
Organophosphates	4	1	5	3	0	2	1	12
Oxygen and ozone	2	1	7	4	2	1	0	9
Peroxides	3	0	6	3	1	3	0	13
Pesticides not otherwise classified	0	0	1	0	0	1	1	2
Phenol compounds	6	0	4	3	1	1	0	6
Phthalates and derivatives	2	2	3	2	0	1	0	7
Polycyclic aromatic hydrocarbons	4	2	5	0	1	1	0	5
Pyrethrins, pyrethrum	0	0	0	0	0	0	0	2
Steroids	0	0	0	2	0	0	0	1
Sulfides and disulfides	2	0	4	2	0	3	0	10
Surfactants	22	10	46	34	9	15	7	81
Terpenes and derivatives	4	4	8	6	6	3	2	15
Volatile anesthetics (except nitrous oxide)	1	1	4	2	1	0	0	6
Zinc compounds	2	0	3	1	0	0	1	4

* Those with "likely" exposure to that particular chemical; "maybe" exposures are excluded. Chemical groups for which there were no exposed subjects are not shown. These groups were acetic acid and derivatives, aluminum, antimony, bipyridyl, carbamate insecticides, aliphatic chlorinated hydrocarbons, aromatic chlorinated hydrocarbons, fluorinated organics, halogenated hydrocarbons not otherwise specified, lithium, nitro compounds, *N*-nitroso compounds, phthalimide fungicides, ribavarin/antivirals, selenium, and tin compounds.

† Corresponds to controls randomly selected from 1987–1988 and serves as referent group for conotruncal and limb cases.

‡ CLP, cleft lip with or without cleft palate; CP, cleft palate.

§ Corresponds to additional controls randomly selected from 1989 combined with 1987–1988 controls and serves as referent group for oral cleft case groups.

ferase T1 (*GSTT1*), *N*-acetyltransferase 1 (*NAT1*), and *N*-acetyltransferase 2 (*NAT2*) (8). We explored genetic variants associated with detoxification enzymes because genetic variation in the metabolic biotransformation of certain chemicals by placenta and embryonic tissues may be an important determinant of embryotoxicity in humans (9–11).

MATERIALS AND METHODS

Study population

Details of this case-control study have been described (12, 13). Included as cases were infants and fetal deaths (≥20 weeks' gestation) diagnosed within 1 year after delivery with conotruncal heart defects, limb deficiencies, or orofacial clefts and born to women residing in most California counties. Eligible were all infants or fetal deaths that were delivered between January 1987 and December 1988 (n = 344,214), except for ascertainment of orofacial cleft cases, which included deliveries through December 1989 (an additional 208,387 infants or fetal deaths considered eligible). Case eligibility was determined by one clinical geneticist (E. J. L.) reviewing detailed diagnostic information from medical records of all hospitals and genetics centers in the surveillance area. Conotruncal heart defect cases were infants and fetuses with anomalies affecting aorticopulmonary septation, including tetralogy of Fallot, dextrotransposi-

tion of the great arteries, truncus arteriosus communis, double outlet right ventricle, pulmonary valve atresia with ventricular septal defect, subaortic ventricular septal defect type I, and aorticopulmonary window. Each case was considered for inclusion and classified after reviewing echocardiography, cardiac catheterization, surgery, or autopsy reports. One or more of these diagnostic procedures was required for inclusion. Limb deficiency cases were infants and fetuses with longitudinal or transverse (including those associated with amniotic bands) deficiency defects of the upper or lower limbs confirmed by clinical examination, radiology, surgery, or autopsy reports. Orofacial cleft cases were those infants or fetuses born with cleft palate (CP) or with cleft lip with or without cleft palate (CLP), each confirmed by clinical description or by surgical or autopsy report. These cases were further phenotypically classified as "isolated" CP, "isolated" CLP, "multiple" CP, or "multiple" CLP on the basis of the nature of accompanying congenital anomalies. CP and CLP cases with no other major anomaly or with anomalies considered minor were classified as isolated. CP and CLP cases with at least one accompanying major anomaly were classified as multiple. Infants diagnosed with single gene disorders, trisomies, or Turner's syndrome (45,X) were excluded.

A total of 972 control infants (652 from the 1987–1988 birth cohort corresponding to the time period for conotruncal and limb cases) were randomly selected from all infants born alive (n = 548,844) in the same geographic area and time period (1987–1989) as cases. Control infants had no major congenital anomalies identified before the first birthday. Telephone interviews were completed with 207 (87 percent) conotruncal case mothers, 165 (82 percent) limb deficiency case mothers, 662 (85 percent) orofacial cleft case mothers, and 734 (76 percent) control mothers. Interviews were completed an average of 3.7 years after the date of delivery for cases and 3.8 years for controls.

Occupational exposure assessment

In addition to information on maternal medical conditions, reproductive histories, and activities associated with various lifestyles, interviews elicited detailed work histories (paid and unpaid) from women for the periconceptional period, defined as 1 month before to 3 months after conception. The requested information included employer name and address, type of industry, period of employment, weekly work hours, job title, and a detailed description of job tasks, including inquiries about materials handled or machines used.

The exposure assessment strategy, described elsewhere, employed an industrial hygienist who characterized occupational activities into tasks, while unaware of whether a woman was a case mother or a control mother (6). A task corresponded to use of a certain machine or process, contact with a commercial chemical product or trade name product, contact with a type of product defined by its end use, behaviors associated with exposures, or working in certain occupational environments. Several information sources were used to determine task-specific exposures, including inquiries to persons working in that industry and Material Safety Data Sheets, available from product manufacturers for hazardous commercial products (14–17).

Information about tasks and task-specific exposures permitted the industrial hygienist to further classify women as "likely" exposed, "maybe" exposed, or "not" exposed. These assignments were made for each of 74 chemical agent groups (table 1). These groups were defined a priori on the basis of potential toxicity and teratogenicity (6). A case mother or control mother could be assigned to multiple chemical agent groups. Assignments of "likely" exposed or "not" exposed, based on tasks, were also made for the following nine end-use chemical groups: dyes and pigments, plastics, propellants, pyrolysis and combustion products, housekeeping cleaners, fungicides, insecticides, herbicides, and other pesticides. A case mother or control mother could be assigned to one or more of the 74 chemical groups and to one or more of the nine end-use groups on the basis of reported tasks.

Analyses

Effects were estimated by the odds ratio, and the precision of the odds ratio was assessed by its 95 percent confidence interval. Analyses estimated the effects of each of the congenital anomaly groups associated with maternal exposure to each of the 74 possible exposures relative to those persons without that particular exposure. That is, an anomaly risk was estimated for a "likely" exposure to one of the 74 chemical groups relative to those persons "not" exposed to that particular group ("maybe" exposed individuals were excluded). Because exposure assessments focused on occupational exposures, analyses were limited to comparisons among women who reported working during the periconceptional period. Considered as covariates for some analyses were maternal race/ethnicity (Latina, foreign born; Latina, US born; White, non-Hispanic; Black; other), education (less than high school graduate; high school graduate; college graduate), and use of multivitamins containing folic acid (in the 4-month periconceptional period, yes vs. no).

Genotyping analyses

Additional molecular genetic analyses were performed on the isolated CLP and isolated CP case groups and a random sample of 300 of the control infants (1987–1989). These analyses involved genotyping each infant for polymorphisms (*GSTMI*, *GSTT1*, *NAT1*, and *NAT2*) of the phase II xenobiotic metabolizing enzymes. Infants' DNA for cases and controls was obtained from newborn-screening blood specimens (12). Infant genomic DNA was extracted from these blood specimens using established methods (18, 19). DNA amplification and restriction fragment length polymorphism analyses followed established procedures available for each enzyme (20–25). All genotyping was done by laboratory staff unaware of the case-control status of the infant.

For *NAT2*, three polymorphisms were analyzed that together account for approximately 95 percent of all slow acetylators; that is, two variant alleles in any combination were considered "slow acetylators" (26). Our working hypothesis was that infants whose mothers were "chemically

TABLE 2. Effect estimates (odds ratios) for selected congenital anomalies associated with periconceptional maternal occupational exposures* to chemical agent groups, California, 1987–1989

Chemical agent groups	Conotruncal heart cases		Limb cases		Isolated CLP† cases		Multiple CLP cases		Isolated CP† cases		Multiple CP cases	
	Odds ratio	95% CI†	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI	Odds ratio	95% CI
Amines, aliphatic							1.5	0.4, 7.1			2.4	0.7, 11.6
Amines, aromatic (including aminophenols)									1.5	0.5, 5.6		
Ammonia and ammonium hydroxide	1.5	0.7, 3.4									2.8	1.2, 7.5
Copper compounds											1.6	0.5, 7.7
Glycol ethers and derivatives											2.2	0.8, 7.0
Hydrocarbons, aliphatic (C_1–C_4)									2.2	0.9, 5.7	1.5	0.5, 7.0
Hydrocarbons, aliphatic (C_5–C_{12})	1.6	0.8, 3.3	1.6	0.7, 3.8								
Ketones									2.3	0.7, 9.1		
Organic dyes (excludes aromatic amines)	5.0	1.3, 16.7			2.7	0.9, 7.7						
Oxygen and ozone							1.8	0.5, 8.8				
Phenol compounds	3.1	0.9, 9.9										
Sulfides and disulfides									1.8	0.6, 6.8		
Terpenes and derivatives			1.6	0.5, 5.5			3.2	1.3, 8.7			1.5	0.5, 7.2

* Effect estimates and chemicals displayed only for those circumstances where there were 10 or more case mothers and control mothers (combined) who were exposed and the odds ratio was ≥1.5. Odds ratios were based on those with "likely" exposure to that particular chemical compared with those without that exposure; "maybe" exposures were excluded.

† CLP, cleft lip with or without cleft palate; CP, cleft palate; CI, confidence interval.

exposed" and who were slow acetylators would be at higher risk for isolated CLP or isolated CP, relative to those infants whose [employed] mothers were not "chemically exposed" *and* who were fast acetylators (i.e., homozygous wildtype or have only one variant allele). For *NAT1* (two gene variants, *NAT1-1088* and *NAT1-1095*), each infant was classified as genetically susceptible (genotype homozygous variant alleles, i.e., *1088A/1088A* or *1095A/1095A*) or not susceptible (genotypes homozygous wildtype or heterozygous variant alleles, i.e., *T1088/T1088*, *T1088/1088A*, *C1095/C1095*, or *C1095/1095A*). For *GSTM1* and *GSTT1*, each infant was classified as genetically susceptible (genotype homozygous variant alleles, i.e., *GSTM1* null or *GSTT1* null) or not susceptible (genotypes homozygous wildtype or heterozygous variant alleles, i.e., *GSTM1* allele present or *GSTT1* allele present). Thus, for these gene variants, our working hypothesis was that infants whose mothers were "chemically exposed" *and* who were genetically susceptible would be at elevated risk for isolated CLP or isolated CP relative to those infants whose mothers were not exposed *and* who were less genetically susceptible.

RESULTS

Among the 1,034 case mothers and 734 control mothers, 565 case mothers and 417 control mothers were employed during the periconceptional period. Among the employed women, 201 case mothers and 158 control mothers were classified as not exposed to any of the 74 chemical groups, 218 case mothers and 146 control mothers were classified as "likely" exposed, and the remainder as "maybe" exposed and excluded from further analyses.

The frequencies of periconceptional maternal occupational exposures to each of the 74 chemical groups are displayed in table 1 for each case group and control group. Odds ratios of 1.5 or greater (based on a minimum of 10 exposed cases and controls combined) were observed for a relatively small number of all the possible exposure-anomaly comparisons. These odds ratios are displayed in table 2. Because of sparse data, many of these other effect estimates were imprecise.

Anomaly risks associated with maternal periconceptional occupational exposures to each of the nine end-use groups are displayed in table 3. Odds ratios of 1.5 or greater were observed for maternal exposures to dyes and pigments (conotruncal defects and multiple CP defects), propellants (isolated CP defects), and insecticides (isolated CP defects and conotruncal defects). Table 3 also displays the results of "any" versus "none" analyses. In general, elevated risks were not observed among infants whose mothers had "any" end-use chemical exposures, relative to those whose mothers had none. Adjustment for maternal multivitamin use, education, and race/ethnicity did not reveal a substantially different pattern of results where data were sufficient to permit comparisons.

TABLE 3. Frequencies and effect estimates (odds ratios)* of selected congenital anomalies associated with periconceptional maternal exposures to chemical end-use groups, California, 1987–1989

"End-use" chemical group	Conotruncal heart cases	Limb cases	Isolated CLP† cases	Multiple CLP cases	Isolated CP† cases	Multiple CP cases
Dyes and pigments						
Frequency (no.)	15	3	14	3	4	4
Odds ratio	2.0	0.6	1.2	0.8	0.9	1.8
95% CI†	1.0, 4.1	0.2, 2.3	0.6, 2.3	0.3, 2.9	0.3, 2.7	0.7, 5.7
Plastics						
Frequency (no.)	24	13	29	6	14	7
Odds ratio	1.2	1.0	1.0	0.7	1.4	1.3
95% CI	0.7, 2.1	0.5, 2.0	0.6, 1.7	0.3, 1.7	0.7, 2.6	0.6, 3.2
Propellants						
Frequency (no.)	7	9	11	3	8	2
Odds ratio	0.6	1.3	0.7	0.7	1.5	0.7
95% CI	0.3, 1.5	0.6, 3.1	0.4, 1.5	0.2, 2.3	0.7, 3.4	0.2, 3.2
Pyrolysis/combustion products						
Frequency (no.)	15	11	13	7	6	
Odds ratio	0.6	0.8	0.5	0.9	0.6	
95% CI	0.4, 1.2	0.4, 1.6	0.3, 0.9	0.4, 2.2	0.3, 1.5	0
Housekeeping cleaners						
Frequency	45	26	65	21	28	13
Odds ratio	1.3	1.1	1.1	1.3	1.4	1.2
95% CI	0.8, 2.0	0.7, 1.9	0.8, 1.6	0.7, 2.2	0.8, 2.3	0.6, 2.4
Fungicides (no.)	1	1	0	0	1	1
Insecticides						
Frequency (no.)	9	2	8	2	5	1
Odds ratio	2.1	0.7	0.9	0.7	1.5	0.6
95% CI	0.8, 5.1	0.2, 3.4	0.4, 2.2	0.2, 3.4	0.6, 4.3	0.2, 4.5
Herbicides (no.)	0	1	1	1	1	0
Other pesticides						
Frequency (no.)	12	5	20	7	7	3
Odds ratio	1.0	0.7	1.0	1.2	0.9	0.7
95% CI	0.5, 2.2	0.3, 1.9	0.6, 1.7	0.5, 2.8	0.4, 2.1	0.3, 2.6
Any above chemical group, yes to any‡						
Frequency (no.)	62	34	89	31	32	18
Odds ratio	1.1	0.8	1.1	1.4	0.9	1.1
95% CI	0.7, 1.6	0.5, 1.4	0.8, 1.5	0.8, 2.4	0.6, 1.5	0.6, 2.2

* Those with "exposure" to that chemical group compared with those with no "exposure" to that chemical group. Odds ratios were not computed for those exposures with fewer than 10 cases and controls combined.

† CLP, cleft lip with or without cleft palate; CP, cleft palate; CI, confidence interval.

‡ Compared with no exposure to all groups.

Analyses of isolated CLP or isolated CP that considered both maternal chemical exposures (74 chemicals) *and* infant-susceptible genotypes are displayed in table 4, for those that produced odds ratios of 2.5 or more. In addition, odds ratios of 2.5 or more for isolated CP were observed for three gene-chemical combinations where the chemical exposures were end-use groups. These combinations were *GSTM1* homozygous variant genotype and plastics (odds ratio (OR) = 2.6, 95 percent confidence interval (CI): 0.8, 7.9), *NAT2* slow acetylator genotype and propellants (OR = 4.0, 95 percent CI: 1.1, 13.5), and *GSTM1* homozygous variant genotype and propellants (OR = 3.4, 95 percent CI: 1.0, 12.0).

DISCUSSION

Overall, our results indicate that maternal occupational exposures to a variety of chemicals in the periconceptional

TABLE 4. Effect estimates (odds ratios) of isolated cleft lip with or without cleft palate and isolated cleft palate among infants with susceptible genotypes for detoxifying enzymes and whose mothers were periconceptionally exposed to chemical groupings, California, 1987–1989

Chemical agent groups and gene*	Isolated CLP†		Isolated CP†	
	Odds ratio‡	95% CI†	Odds ratio	95% CI
Drugs, not otherwise classified				
GSTT1			4.9	0.5, 32.2
Hydrocarbons, aliphatic (C_1–C_4)				
NAT2	4.0	0.5, 19.3	2.5	0.3, 24.9
GSTM1			4.6	1.0, 19.0
GSTT1			3.2	0.7, 13.0
Iodine compounds (organic and inorganic)				
GSTM1			6.5	0.7, 42.5
GSTT1			2.7	0.2, 28.0
Ketones				
GSTM1	2.5	0.3, 15.9	6.9	0.7, 44.9
NAT2			2.5	0.2, 24.4
Methacrylates and related compounds				
GSTM1			3.2	0.3, 31.7
Organic acids, derivatives, not otherwise classified				
GSTT1			7.2	0.7, 42.1
Organic dyes (excludes aromatic amines)				
GSTM1	2.5	0.3, 15.9		
Oxygen and ozone				
GSTM1	4.0	0.5, 21.6		
Phenol compounds				
GSTM1			3.4	0.3, 33.1
Phthalates and derivatives				
NAT2			2.5	0.3, 24.6
Polycyclic aromatic hydrocarbons				
GSTM1			3.2	0.3, 31.5
Sulfides and disulfides				
GSTT1			3.6	0.6, 19.3
NAT1-1095			2.7	0.6, 12.7
Terpenes and derivatives				
GSTM1	2.6	0.3, 16.5	3.6	0.4, 35.6

* *GSTM1*, glutathione-*S*-transferase M1; *GSTT1*, glutathione-*S*-transferase T1; *NAT2*, *N*-acetyltransferase 2.

† CLP, cleft lip with or without cleft palate; CP, cleft palate; CI, confidence interval.

‡ Reflects estimate of effect for those women who worked, had the given exposure, and whose infants had the susceptible genotype, compared with those women who worked and did not have that given exposure and whose infants did not have the susceptible genotype. Susceptible genotype was defined as follows: for *NAT2* as slow acetylators and for *NAT1* (*1088* and *1095*), *GSTM1*, and *GSTT1*, based on the following allelic variants: *1088A*/*1088A*, *1095A*/*1095A*, *GSTM1* null, or *GSTT1* null.

period do not contribute substantially to the risk of conotruncal heart defects, limb deficiencies, or specific oral cleft phenotypes in this California population. These data revealed a few potential associations between anomaly risk and maternal exposure to chemicals, as well as exposure and infant-susceptible genotype combinations. However, many of these associations were imprecise because of the small number of chemical-exposed individuals and could have arisen by chance as a result of the relatively large number of comparisons made. The unique aspects of this population-based, relatively large, case-control study are as follows: 1) we investigated an array of chemical exposures assessed by an industrial hygienist from task-specific information rather than relying on maternal reporting of specific chemical exposures or inferences from occupational title alone; 2) we focused effect estimation on specific subgroupings of

congenital anomalies rather than lumping together anomalies that might be pathogenically unrelated; and 3) for isolated CLP and isolated CP, we investigated maternal exposures in combination with potentially susceptible infant genotypes of chemical detoxification enzymes.

Other investigators have observed elevated risks in some studies that have focused on maternal occupations or maternal occupational exposures as risk factors for the specific congenital anomalies that we studied. Elevated risks have been reported, although not in all studies, with "exposures" such as working as a hairdresser (27, 28), working in leather or shoe manufacturing (27), working in transport and communication (29), working in jobs that require standing (30), working as a housekeeper (28), and more specific exposures such as organic solvents (31–34), glycol ethers (28, 35), detergents (32), disinfectants (32), aliphatic compounds (28), lead (28), biocides (28), antineoplastic drugs (28), and trichloroethylene (28). Only a few studies, however, have investigated detailed maternal occupational exposures as risk factors for the specific congenital anomalies included here. The studies whose exposure assessments most closely resemble ours are those of Cordier et al. (35) and Lorente et al. (28). Each of these studies observed elevated risks of oral cleft phenotypes (and other congenital anomaly phenotypes in the study by Cordier et al.) associated with maternal exposures to glycol ethers. Lorente et al. (28) further observed elevated risks of cleft lip with or without cleft palate among mothers exposed to lead, biocides, antineoplastic drugs, and trichloroethylene, and they observed elevated risks of cleft palate among mothers exposed to aliphatic acids. Our study observed elevated risks (i.e., >1.5) for cleft palate but not for cleft lip with or without cleft palate, for some of these exposures (aliphatics and glycol ethers) (table 2), although the effect estimates were imprecise. The approach by Lorente et al. (28) was more detailed in its exposure assessment in that, for some jobs, supplemental questionnaires were used. This approach likely resulted in more detailed information about the exposures of interest than was available in our study.

Our study explored the potential effects of genetic variants of detoxification enzymes because variation in the metabolic biotransformation of certain chemicals by embryonic tissues may be an important determinant of embryotoxicity in humans (9–11). Detoxification first involves phase I enzymes that metabolically activate toxins to highly electrophilic intermediates, many of which have been demonstrated to be carcinogenic, mutagenic, or teratogenic (36, 37). In the second detoxification step, phase II enzymes metabolize reactive intermediates and transform them to more excretable, hydrophilic products. An enzyme variant that produces a slower clearance of reactive metabolites might lead to increased concentrations of reactive intermediates or to increased length of fetal exposures. To relate this to the potentially toxic effects of maternal chemical exposures, a fetus may be more susceptible to the effects of such exposures if it produces variant forms of these enzymes with altered kinetics. *GSTT1*, *GSTM1*, *NAT1*, and *NAT2* were selected as candidate genes for study because of their role in phase II chemical detoxification. For instance, *NAT1* and *NAT2* encode for phase II enzymes that catalyze detoxification of aromatic and heterocyclic amine and hydrazine toxins (26). For both genes, polymorphisms associated with altered enzyme activity and with variation of DNA adduct levels have been identified (38–41), and the presence of these polymorphisms has been associated with increased risk for a variety of human cancers (26, 38, 39, 42–44). Although some evidence exists indicating that these enzymes are expressed in the fetus early in gestation (45), maternal enzyme variants would also be of potential importance in terms of risk. Unfortunately, maternal DNA was not available for analyses.

This study is further limited by aspects pertaining to its exposure assessment, a common limitation of case-control studies that investigate the effects from occupational and environmental exposures. One limitation was that women who were considered to have an exposure to a particular chemical agent group or end-use group were all analytically treated as if they had the same level and frequency of exposure. This assumption, if false, would likely lead to attenuated effect estimates. No surrogates of exposure dose were available. Another potential limitation is that all exposure categorizations were made by one industrial hygienist. Although the industrial hygienist was unaware as to whether a woman was a case mother or a control mother, we did not evaluate the exposure designations she made for each reported task against the designations of another hygienist. Benke et al. (46) observed that the use of a panel of experts, versus a single expert, may reduce the impact of exposure misclassification on estimated risks. The relative benefits of such an approach have been discussed by others as well (47). Benke et al. (46) also observed that interrater agreement between experts was low if the exposure prevalence was less than approximately 8 percent, suggesting the difficulty in assigning exposure categorizations for low prevalence exposures. Prevalences were uncommon for many of the occupational exposures we considered. The "expert" assessment approach, similar to the one used here, however, has been argued to be the method of choice for assessing occupational risks in case-control studies (7). Fritschi et al. (48) discuss the merits of this approach. An additional potential limitation is that the number of women considered "exposed" (likely exposed) was modest, even though the study populations were relatively large. Numbers of available subjects were even more sparse in analyses that attempted to estimate the effects associated with gene-exposure combinations.

Another potential limitation is the possibility that the depth of probing for occupational activities and the quality of respondent recall were reduced. Some indirect evidence for this was the observation that the proportion of case women and control women in this study considered not exposed was substantially larger than the number of "not exposed" in an earlier study we performed (3). The methods of obtaining occupational activities between the two studies varied. In the other study, interviews were performed in person an average of 6 months after delivery, whereas in this study the information was collected over the telephone an average of 4 years after delivery. This difference may be indicative of misclassifying women as not exposed when they should have been classified as exposed. Assuming these

errors were unrelated to case or control status, the resulting bias attenuated estimated effects.

Despite these limitations, a few clues have been identified that may prove worthy of additional investigation. However, this study also highlights that specific exposures are infrequent, making it difficult to study such relations. This particular challenge is further amplified for analyses attempting to describe how gene variants modify risk. These complexities showcase the need for even larger studies than the current one to appropriately address these questions of anomaly risk. A more reassuring view, however, is that the infrequency of exposures, if real, would tend to suggest that the population burden of specific anomalies that can be attributed to specific chemical exposures is likely to be modest.

ACKNOWLEDGMENTS

This work was partially supported by the Cigarette and Tobacco Surtax Fund of the California Tobacco Disease Research Program (grants 1RT466, 3RT0413, and 6RT0360), University of California; by grant RO1 DE 12898 from the National Institutes of Health; by funds from the Centers for Disease Control and Prevention (Centers of Excellence award no. U50/CCU913241); and by the US Environmental Protection Agency's Science to Achieve Results Program (grant 82829201).

The authors are grateful to Elizabeth Katz for her categorizations of exposures.

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