Prenatal exposure to glycol ethers and response inhibition in 6-year-old children: The PELAGIE cohort study

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Abstract

Background: Exposure to glycol ethers (GEs) is suspected of impairing neurodevelopment in children, but the specific impact on their inhibitory capacity, a central deficit of ADHD, has never been studied. We aimed to assess the impact of prenatal exposure to GEs on the response inhibition of children aged six years. **Methods:** In total, 169 mother-child pairs from the French cohort PELAGIE (2002-2006) were studied. Maternal urinary concentrations of six GE metabolites (alkoxycarboxylic acids) were measured during pregnancy. Multiple imputation by quantile regression was used to handle non-detected values and the data were then classified into quartiles. Inhibition of children was evaluated by the Rhythmic Continuous Performance Test 90 (R-CPT90). The inhibition score (percentage of correct responses to non-target stimuli) was corrected for compliance with the instructions (percentage of correct responses to target stimuli). The analysis used a multiple linear regression model, adjusting for confounding factors for each metabolite. **Results:** Median concentrations of metabolites ranged from 0.02 mg/L (Ethoxyacetic acid, EAA) to 0.39 mg/L (Phenoxyacetic acid, PhAA). The median corrected inhibition score was 37.9% [first quartile: 29.8 - third quartile: 47.9]. We found a negative and statistically significant association between the inhibition score and prenatal urinary EAA concentration (p-trend = 0.03), with a significant β coefficient for the third quartile (β= -0.064; 95% confidence interval: -0.121, -0.007). There were no statistically significant associations for the other five metabolites. **Conclusion:** These results are consistent with the hypothesis of possible impact of prenatal environmental exposure on inhibitory capacity among children. Data about the GEs metabolized to EAA (history of exposure sources and toxicokinetics) should be gathered to further interpret these results and guide precautionary measures.

Keywords: solvents, glycol ethers, neurodevelopment, response inhibition, prenatal exposure

Background

Inhibitory capacity underlies various paradigms concerning the deletion of inappropriate cognition and behaviors (1). Its behavioral manifestation, response inhibition *(i.e.* resistance to impulsivity), is its most easily observable component via objective experimental tasks (1). Inhibitory capacity influences working memory, on which learning depends. Its absence has been identified as a core deficit of attention deficit disorder, with or without hyperactivity (ADHD) (2), the most common disorder of school-aged children, with a worldwide and French prevalence ranging between 2 and 7%, depending on the study (3). Several studies have shown that prenatal exposure to neurotoxic agents, such as polychlorinated biphenyls (PCBs), organochlorine pesticides (4,5), and methylmercury (6), can alter attention and response inhibition, increasing the risk of ADHD in children.

Glycol ethers (GE) are oxygenated solvents. Due to their amphiphilic property, they have been introduced into the composition of many industrial and domestic products, such as water-based paints, ink, glue, cleaning products, liquid soaps, cosmetics, and even some pharmaceutical products. Annual usage of GEs was estimated to be 350,000 tons in Europe in 2006 (7). Consequently, the general population, including pregnant women, has had regular, frequent contact with GEs since the 1960s (8–11). Exposure to GEs occurs primarily via inhalation and dermal routes. In humans, ethylene-containing GE parent compounds are readily metabolized by aldehyde and alcohol dehydrogenase to alkoxycarboxylic acids. These metabolites are recognized to be responsible for the toxic effects of GEs and their measurement in urine samples is considered to be the method of choice for biomonitoring (12).

Organic solvents can cross the placenta and the fetal blood-brain barrier. Several experimental studies have documented evidence of behavioral changes and neurochemical modifications in young rats after maternal exposure to two GEs (ethylene glycol monomethyl ether (EGME) and ethylene glycol monoethyl ether (EGEE)) at concentrations that produced no observable maternal toxicity (13). Seven studies have examined the effect of prenatal exposure to organic solvents on neurodevelopment in humans. Five reported that maternal occupational exposure during pregnancy was associated with lower scores for visuospatial and graphomotor ability (14), visual ability (14,15), and neurobehavioral performance (16,17), with higher scores of externalizing behavioral disorders among two-year-old children (attenuated by age six) (18). An older study found that children exposed in utero walked earlier and observed no association with behavioral problems (19). For GE, previous findings of the French PELAGIE cohort have shown lower Wechsler Intelligence Scale for Children (WISC-IV) Verbal Comprehension Index scores and lower visuo-spatial ability of the NEPSY (Neuropsychological Assessment) Design Copying subscale scores at six years of age, associated with prenatal urine concentrations of two GE metabolites (phenoxyacetic acid (PhAA) and ethoxyacetic acid (EAA), respectively) (20). Given such central nervous system involvement, it is possible that prenatal GE exposure alters response inhibition in children.

The aim of this study was to investigate associations between prenatal exposure to GEs, as measured by urine GE-metabolite concentrations, and the response inhibition of six-year-olds, using the French PELAGIE cohort data.

Methods

Study population and design

The PELAGIE mother–child cohort study, described previously (20,21), enrolled 3,421 pregnant women from Brittany, France between 2002 and 2006. Women were recruited before the 19th week of gestation (median, 10 weeks; minimum–maximum, 4–17 weeks) by their gynecologist, obstetrician, or sonographer at the first visit of the pregnancy.

At inclusion, pregnant women completed a self-administered questionnaire about their family, diet, and lifestyle. Throughout the follow-up at age 2 and 6, additional self-administered questionnaires about their sociodemographic characteristics, housing characteristics, and children's health and behavior were completed.

A subcohort of 591 mother–child pairs was randomly selected from the live-born singleton children for a neuropsychological assessment at six years of age (maximum: six years + three months). Twenty pairs were excluded because of preterm birth, neonatal respiratory distress, hospitalization, or Down syndrome, 125 could not be reached by telephone, and 18 were further excluded because the child had previously undergone neuropsychological or behavioral tests (to avoid bias due to the learning effect). Among the 428 remaining families, 287 (67%) mothers agreed to participate with their child. Neuropsychological follow-up began in September 2009 and ended in October 2012.

Assessments were conducted at home by two trained psychologists, blinded to the prenatal exposure. One performed the children’s neuropsychological assessments, while the other tested the mother’s Verbal Intelligence Quotient (using the Wechsler Adult Intelligence Scale, 3rd revision-WAIS-III) (22) and assessed the home environment (quality and extent of stimulation available to the child in the home environment) using the HOME inventory (Home Observation for Measurement of the Environment) (23). Assessments lasted a total of three hours in the morning, including breaks. The two psychologists were supervised by four pediatric neuropsychologists in meetings held every two months.

The child neuropsychological assessment was intended to cover his/her cognitive functions such as memory, attention/inhibition, vocabulary, eyesight, and behavior (20). Response inhibition was initially assessed using Conners' Continuous Performance Test (CPT II), but it was soon replaced by a shorter test, the Rythmic Continuous Performance Test 90 (R-CPT90). This test was administered to all children just after the mid-assessment break.

Finally, the 169 mother–child couples with both available prenatal urinary samples (71%) analyzed for GE-metabolite measurements at the LABOCEA laboratory (Plouzané, France) and available children R-CPT90 tests (82%), were included in the analyses.

Assessment of response inhibition

The Rythmic Continuous Performance Test 90 (R-CPT90) was initially calibrated for 7½-year old children (6, unpublished manuscript “Description of the Rythmic continuous Performance Test 90”, F. Debes, 2009). In contrast to most CPT tests, designed to be maximally sensitive to sustained attention, the R-CPT90 test uses high target-stimuli presentation rates that are maximally sensitive to response inhibition (6,24).

It runs for seven minutes and presents 200 stimuli, at 1,200 ms intervals, in the form of one-digit numbers presented on a computer screen, accompanied by a short simultaneous auditory signal. Rythmic responses to an audible beep were required between the stimuli to reduce the trade-off between speed and accuracy. Target stimuli, 90% of all stimuli (180 in total), were the number nine (“9”), and non-target stimuli, the remaining 10% (20 in total), were all other numbers between “0” and “8”. The child is instructed to react to target stimuli by pressing the space bar on the keyboard. For non-target stimuli, reactions should be inhibited and held back; the success in doing so is the main outcome-measure of the test. A practice trial of 10 stimuli was carried out before the start of the test to ensure that the test procedure was well understood.

Correct responses to target stimuli reflect compliance with the test procedure, the threshold of 70% being considered acceptable compliance. The endpoint used to reflect response inhibition in the current analysis is thus the percentage of correct responses to non-target stimuli (reflecting inhibition ability) multiplied by the percentage of correct responses to target stimuli (reflecting compliance). Without this correction, non-participation for whole or part of the test would artificially and falsely inflate the success rate of response inhibition for non-target stimuli. The score obtained was expressed as a percentage, a high score indicating inhibitory capacity.

During the performance of the R-CPT, we noted coding inconsistencies for the stimulus type and thus excluded responses to the corresponding three stimuli (three target stimuli, same for all children). The analyses were thus based on 177 target stimuli and 20 non-target stimuli.

Chemical analyses

Six maternal urinary GE metabolites were measured: methoxyacetic acid (MAA), ethoxyaceticacid (EAA), ethoxyethoxyacetic acid (EEAA), 2-butoxyacetic acid (BAA), phenoxyacetic acid (PhAA), and 2-methoxypropionic acid (2-MPA). The parent compounds of these metabolites and their sources at the time of inclusion are summarized in Table 1 (7).

At inclusion, each woman mailed a first morning void urine sample in a 10-mL test tube (95 × 16-mm polypropylene, with wing plug; nitric acid was used as conservative). Samples were sent in a rigid opaque box at ambient temperature. Upon arrival, urine samples were frozen at –20°C until analysis. The median time from urine-sample collection by a participant to receipt of the sample by the study laboratory was two days (IQR, 1–3; minimum–maximum, 0–27), and the median duration of storage 103 months (IQR, 97–110; minimum–maximum, 84–116). The samples were analyzed by gas chromatography (HP 7890A; Agilent, Santa Clara, CA, USA) and triple quadrupole mass spectrometry (HP 7000C; Agilent), with a Varian Factor

Four VF-1ms capillary column (15 m × 0.25 mm, 0.1 μm; Agilent), as described elsewhere (20). The limit of detection (LOD) was 0.003 mg/L for all metabolites.

Statistical analyses

Data imputation

Values below the LOD accounted for 0–10% of the values for the GE urinary metabolites. They were imputed by quantile regression imputation of left-censored data (QRILC) multiple imputation, with the function *impute.QRILC* of the *imputeLCMD* package of R Studio software (25,26). This method, designed for left-censored data, imputes missing elements by randomly drawing from a truncated distribution with parameters estimated using quantile regression. Beforehand log-transformation was performed to improve imputation accuracy. Five complete datasets were generated. The few missing values for covariates (< 5%) were replaced by the modal value.

Associations between inhibition and GE metabolite concentrations

The following potential confounding variables were assessed, chosen based on prior knowledge of factors that influence neurodevelopment and on previous examinations from another birth cohort in the Faroe Islands (6,27,28): *a*) *maternal characteristics at inclusion (< 19 weeks of gestation):* mothers' age (continuous), body mass index (< 18.5, [18.5-25.0[, [25.0-30.0[, or ≥ 30.0 kg/m^2), tobacco use in early pregnancy (yes or no), usual consumption of fish before pregnancy (≥ 2 portions per week or less), parity (0 or ≥ 1 child), mother's educational level (≤ 12 years or > than 12 years); *b)maternal characteristics at the six-year follow-up:* maternal WAIS-IQV score (quartiles); *c*) *family characteristics at the six-year follow-up:* HOME score (quartiles), total number of cigarettes smoked per day at home (0, ≤ 10, > 10 cigarettes), number of children at home (1, 2, 3, ≥ 4), residence (rural or urban); *d) children's characteristics:* gender (male, female), gestational age at birth (weeks, continuous), birth weight (g) (continuous), duration of breastfeeding (no, ≤ 16 weeks, or > 16 weeks),

urinary cotinine detection in child (> 6 µg/L) (yes, no), acidic-basic lead exposure (terciles: measured as acid-leachable lead in living room floor dust collected at the six year visit (using a standard protocol for dust sampling: ASTM E1792-03–Standard Specification for Wipe Sampling Materials for Lead in Surface Dust (29) (terciles), school (nursery or primary school), duration of sleep (< 10h30, 10h30-11 h, > 11h), duration of videogame playing (no videogames, < 1h30, ≥ 1h30 per week), duration of TV watching (< 2h30, 2h30-4h30, > 4h30 per week), regular extra-curricular sports (yes, no); and *e) Inhibition assessment:* the psychologist who administered the psychological tests to the child (n°1 or n°2).

For each GE metabolite, covariates were introduced into the multivariable models if they were associated with both the inhibition score and urinary concentrations (continuous, log-transformed) with a p-value < 0.20. Pooled results from the five complete datasets were obtained by applying Rubin rules for multiple imputation (30). A previous study based on the PELAGIE cohort (31) showed a potential impact of sampling conditions on GE metabolite concentrations. Thus, the supplementary following covariates were *a priori* forced into all models: mother's urinary creatinine concentration (continuous, log-transformed) to account for urine dilution, storage time between sample recovery and analysis (< 99.4, 99.4-109.5, > 109.5 months), gestational age of the mothers at inclusion (< 10, 10-13, >13 weeks), transport time at room temperature (< 2, 2-3, > 3 days), and sampling period (Tuesday to Saturday *versus* Sunday or Monday).

Multivariable linear regression models were generated to investigate the associations between the inhibition score of six-year-old children and the GE metabolite concentrations in maternal prenatal urine samples. First, urinary metabolite concentrations were categorized in quartiles, with no prior assumption about the shape of the relation with the inhibition score. P-trends using the same models with quartiles of concentrations recoded into numerical values (1 to 4) were also calculated. Second, we assessed the monotonic trends of metabolite concentrations

with inhibition scores in full multivariable models in two ways: graphically (mean inhibition score value estimated by the full model in each quartile, according to quartile) and with the log-likelihood ratio test, comparing models that included the metabolite concentrations in quartiles as a categorical variable to models that included the concentrations in quartiles as a continuous variable. The assumption of linearity was graphically rejected and the use of continuous data did not improve the log-likelihood (log likelihood ratio test not statistically significant). Thus, we decided not to use the concentration data as a continuous variable in the model.

P-values < 0.05 were considered statistically significant, and all tests were two-sided. All analyses were performed with R software (version 3.5.0).

Ethics Statement

All adult participants provided written informed consent. Children provided verbal and witnessed assent. This study was approved by the French Consulting Committee for the Treatment of Information in Medical Research (no. 09.485) and the French National Commission for the Confidentiality of Computerized Data (no. 909347).

Results

Study population

The characteristics of the 169 mother–child pairs are summarized in Table 2. The characteristics of this sample were quite similar to those of the initial population of the PELAGIE cohort (see Additional file 1: Table S1). Women included in the current study were approximately the same age (median age 30.8 *vs* 29.9 years), tended to have a higher educational level (64.5% *vs* 62.4%), to be more often smokers during pregnancy (45.6% *vs*

28.2%), and slightly more overweight (18% *vs* 17%) and less nulliparous (38.5% *vs* 44.5%). They ate fish once a week or less before conception (67.5%).

The sex-ratio of the children was 1. The six-year-old children predominantly attended nursery school and participated in regular extra-curricular sports. Only 4% were single children and 54% of families lived in rural areas.

R-CPT90 results

The results of the R-CPT90 tests are presented in Table 3. Among all children, 118 (69.8%) obtained > 70% correct responses to target stimuli. The median response inhibition score, corrected for compliance, was 37.9% [first quartile (Q1): 29.8 – third quartile (Q3): 47.9; minimum: 4.8 - maximum: 76.4].

Urinary metabolite concentrations

Detection frequencies of the six GE metabolites measured in the prenatal urine samples ranged from 90.5% (EEAA) to 98.8% (PhAA) and the median concentrations ranged from 0.016 mg/L (EAA) to 0.388 mg/L (PhAA) (Table 4).

Association between GE metabolite concentrations and response inhibition.

We found a negative and statistically significant association between the response inhibition score and the prenatal urinary concentration of EAA (p-trend = 0.03), with a statistically significant β coefficient for the third quartile (β = –0.064; 95% confidence interval (CI): –0.121, –0.007), and a similar β coefficient for the fourth quartile, close to the statistical significance (β = –0.005; 95% CI: –0.117, 0.006) (Table 5). Excluding seven outliers (using studentized residuals) did not alter the results and the statistical significance remained (p-trend = 0.03). There was no consistent association for the five other GE metabolites. Five to nine covariates were included in the models as potential confounding factors (Table 5).

Discussion

We found that higher prenatal urinary concentration of the EAA metabolite is associated with lower response inhibition score among six-year-old children: half of the children with the highest concentrations had a performance reduction of 6% compared to the quarter of children with the lowest exposure levels. No association was observed for the five other GE metabolites of interest.

Our study had several strengths. It drew upon the PELAGIE mother-child cohort, purposely designed to explore the impact of prenatal chemical exposure on child development (17,21,32). Data on a large set of potential confounding factors was collected and considered in statistical analyses when necessary. Exposure was estimated using concentrations of GE metabolites measured in urinary samples collected during the first trimester of pregnancy to estimate prenatal exposure to GEs from all sources. We carried out a creatinine correction to adjust for urine dilution when examining biomarkers. Concentrations were also adjusted for gestational age, as pregnancy causes variations in creatinine metabolism and excretion, which can modify urinary dilution. We used a form of the CPT test that uses a high proportion of target stimuli, as previously used in studies evaluating response inhibition (6,24 and unpublished manuscript, F. Debes, 2009), to assess the outcome. Finally, the observed association is unlikely to be a chance finding considering the trend observed across categories.

This study also had several limitations. A single measurement of GE metabolites cannot account for intra-individual variability over time, given their rapid clearance in humans, from 6 to 80 hours (7), and potential variation in exposure intensity over time. There is yet no available data on the within- and between-subject variability of the urinary concentrations of GE metabolites. The sample of the first morning urine may mainly reflect part of the previous

day's exposure. However, most of the GEs of interest are included in the composition of cosmetics or cleaning products, for which we assume a regular use across days or weeks by the women. Only a few studies have reported on the determinants of the urinary concentrations of these GE metabolites. Two recent French surveys found that urinary concentrations of PhAA and EEAA were correlated with uses of cosmetics in both domestic (on the day of use and the day before) or occupational (regular use) context (10,33). EAA urinary detection was associated with occupational use of cleaning products (10) but possible domestic exposure sources were not considered. Besides, BAA urinary concentrations were mainly associated with occupational uses of glues, paints, cosmetics and cleaning products (10,33). Furthermore, we cannot exclude the possibility that our finding could be explained by one of the various chemicals used in combination with GEs. No adjustment for exposure to solvents later in pregnancy or during childhood was performed in this study, whereas these types of exposure can also impair brain function, including working memory and attention (34-36).

Concerning the assessment of response inhibition, the R-CPT90, a short version of the CPT with only 20 non-target stimuli (*vs* 36 to 60 in CPTs used in previous studies (6,24)), may have lacked sensitivity to reveal more subtle modifications in response inhibition associated with other metabolites. Moreover the test was designed for children aged 7½ years, whereas the current study population of six-year-olds could differ in learning achievement, including the ability to distinguish mirror numbers, such as "9" *vs* "6" (37), leading to falsely incorrect responses. Indeed, the mean uncorrected score in response inhibition in our study was lower than that in a previous study using this test (mean uncorrected score: 52% *vs* 70% and mean corrected score: 39% *vs* 63%) (unpublished manuscript, F. Debes, 2009). In addition, we observed relatively poor compliance of the children with the test; 30% of children *vs* approximately 3% in a previous study (unpublished manuscript, F. Debes, 2009) had a

percentage of correct responses to target stimuli below 70%, the threshold considered to be acceptable compliance. This can be explained by the duration of the entire test battery of three hours, a long time for young children. However, the score was corrected to take this into account.

The high frequency of detection of urinary GE metabolites in our study (90.5% to 98.8%) and their relatively short half-lives suggest that exposure was frequent and repeated. The median concentrations, ranging from 0.016 mg/L (EAA) to 0.388 mg/L (PhAA), are close to the median values found in previous studies conducted in the general populations of Germany (2007-2008) and of the North of France (2008-2009), with one exception. The PhAA concentration was two times higher compared to our study population (0.80 mg/L and 0.70 mg/L in Germany and North of France, respectively) (9,33).

Previous studies have identified neurophysiological mechanisms involved in the neurotoxic effects of GE. Experimental studies have shown that prenatal exposure of rats via inhalation to 100 ppm ethylene glycol ethyl ether (EGEE), a precursor of EAA, and 25 ppm ethylene glycol monomethyl ether (EGME) had teratogenic effects. It was also associated with behavioral impairment (learning activity, circadian activity, and neuromuscular ability) and changes in the concentrations of four neurotransmitters (acetylcholine, dopamine, norepinephrine, and 5-hydroxytryptamine (serotonin)) in the brains of young rats at concentrations which produced no observable effects in the maternal animals (13,38). Neuropharmacological studies have shown that neurotransmission, especially dopaminergic and noradrenergic, modulate inhibition (1).

Only one study has assessed the specific impact of prenatal exposure to GE on child neurodevelopment in humans. This study, also conducted on the PELAGIE cohort, highlighted an association between prenatal urinary concentrations of EAA and impaired

visuospatial ability assessed by a NEPSY Design Copying subtest (20) in six-year-old children. This study also found that prenatal urinary concentrations of PhAA were associated with an alteration of the WISC verbal comprehension subscale (ability to think, understand, conceptualize, and categorize), but not the WISC working memory subscale (WISC-WMI) (ability to concentrate and manage information). As the WISC-WMI is closer to the inhibition capacity domain analyzed in the former study, the absence of an association between PhAA concentrations and response inhibition observed in the current study are consistent with the previous results.

Our results are consistent with those of two studies (17,18) on the association between ADHD in children and exposure to organic solvents during pregnancy, although they were not specific to GE but extended to organic solvents in general in occupational settings. Both studies were based on the same PELAGIE cohort but assessed the exposure differently (from maternal self-reports collected at the beginning of pregnancy). Pelé *et al.* (17) reported higher scores of attention deficit/hyperactivity and aggression among two-year-old children in association with higher level of maternal occupational exposure to organic solvents. Costet *et al.* (18) highlighted statistically significant higher scores of externalizing behavior (highly related to the attention/deficit hyperactivity score) at two years of age and a non-significantly higher externalizing behavior score (highly related to conduct problems and the hyperactivity score) at six years of age (higher scores indicating more potential problems), associated with maternal occupational exposure to organic solvents during pregnancy.

The GE metabolite EAA has a half-life of approximately 24 to 42 hours and five parent compounds as follows: ethylene glycol ethylether (EGEE), ethylene glycol diethylether (EGDEE), diethylene glycol ethylether (DEGEE), diethylene glycol diethylether (DEGDEE), and triethylene glycol ethylether (TEGEE). In the early 2000's, the use of EGEE was prohibited because of its effects on reproduction (fetotoxicity and teratogenicity) (7). At the

time of urine sampling (2002-2006), the two most encountered precursors were DEGEE and TEGEE, known to have adverse health effects (acute toxicity and effects on reproduction) (7,39). DEGEE was one of the main GEs used in cleaning products, paints, inks, cosmetics, and drugs in France. Its use is now subject to restrictions in cosmetics. TEGEE was mainly used in paints (7). There are no data available on the use of EGDEE and DEGDEE at the time of sampling. The use of EGDEE has since been prohibited (7). The parent compounds of EAA most likely to be involved in the observed effect on response inhibition were therefore DEGEE, TEGEE, EGDEE, and DEGDEE. Two GEs (DEGEE and TEGEE) are also parent compounds of EEAA, but EAA or EEAA metabolic fractions in urine excretion after a contact with a parent compound are unknown. The latter did not show any association with response inhibition ($p = 0.66$), with a median concentration twice as high as that for EAA (0.028 mg/L *vs* 0.016 mg/L). However, this contrast cannot be further explained because of the lack of data on tonnage, exposure, and metabolic fractions.

Conclusion

This study supports the hypothesis of an impact of prenatal environmental exposure on the inhibitory capacity of children, as it shows that high urinary concentrations of EAA during the first trimester of pregnancy are associated with low response inhibition scores among six-year-olds. Four GEs (DEGEE, TEGEE, EGDEE, and DEGDEE) are suspected to be involved. However, more knowledge is needed on GE (exposure sources, toxicokinetics, metabolites, underlying biological mechanisms that might explain the association with neurodevelopment) to further interpret these results and potentially guide precautionary measures.

List of abbreviations

ADHD: attention deficit disorder, with or without hyperactivity

BAA: 2-butoxyacetic acid

CPT: Conners' Continuous Performance Test

DEGDEE: diethylene glycol diethylether

DEGEE: diethylene glycol ethylether

EAA: ethoxyacetic acid

EEAA: ethoxyethoxyacetic acid

EGDEE: ethylene glycol diethylether

EGEE: ethylene glycol monoethyl ether

EGME: ethylene glycol monomethyl ether

GE: glycol ethers

HOME: home observation for measurement of the environment

LOD: limit of detection

MAA: methoxyacetic acid

MPA: 2-methoxypropionic acid

PCBs: polychlorinated biphenyls

PhAA: phenoxyacetic acid

QRILC: quantile regression imputation of left-censored data

Q1: first quartile

Q3: third quartile

R-CPT90: Rhythmic Continuous Performance Test 90

TEGEE: triethylene glycol ethylether

WAIS-III: Wechsler Adult Intelligence Scale, 3rd revision

WISC: Wechsler Intelligence Scale for Children

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on request and after examination according to the French ethical rules and the European individual data protection regulations.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AR analyzed and interpreted the data and drafted the manuscript.

RG contributed to the interpretation of data.

AL participated in the conception of the study and data collection and contributed to the interpretation of data.

FR participated in the conception of the study, data collection, and their quality control.

CW performed the data management and contributed to interpretation of the data.

CM has coordinated the data since the beginning of the cohort.

FLG performed the chemical analyses.

SC initiated the cohort and contributed to interpretation of the data.

JFV contributed to the conception of the study, analysis of data, and writing of the manuscript.

CC conceived and planned the study, contributed to the analysis of data, writing and revision of the manuscript.

All authors read and approved the final manuscript.

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Table 1. Parent compounds and sources of the six GE metabolites during the inclusion period (2002-2006, France).

Glycol ether (parent compound)	Alkoxycarboxylic acid (metabolite)	Major sources according to AFSSET, 2008	Date of banning
EGME	MAA	Aeronautics	April 19, 2001*
EGDME	MAA	X	October 28, 2004*
DEGME	MAA	Household and industrial cleaning products / Plant protection products	-
TEGME	MAA	Industrial cleaning products	-
TEGDME	MAA	Household and industrial cleaning products	October 28, 2004*
EGEE	EAA	Metalworking sector / Rubber and plastics industry / Cleaning and printing	April 19, 2001*
EGDEE	EAA	X	-
DEGEE	EAA ; EEAA	Paints / Inks and household products / Biocides / Drugs / Cosmetics	Restriction in cosmetics on November 23, 2005
DEGDEE	EAA	X	-
TEGEE	EAA ; EEAA	Paints	-
EGBE	BAA	Paints / Varnishes / Inks / Household and industrial cleaning products / Biocidal products / Plant protection products / Cosmetics	Restriction in cosmetics on November 23, 2005
DEGBE	BAA	Paints / Varnishes / Inks / Household and industrial cleaning products / Biocidal products / Plant protection products / Cosmetics	Restriction in cosmetics on November 23, 2005
TEGBE	BAA	Varnishes	-
EGPhE	PhAA	Cosmetics	Restriction in cosmetics on November 23, 2005
PGME	2-MPA	Products for professional use (all sectors) / Domestic use (metal, varnishes, household products)	-

X: no use identified in France during the inclusion period;

*Date of decree;

EGME: ethylene glycol methyl ether; EGDME: ethylene glycol dimethyl ether; DEGME: diethylene glycol methyl ether; TEGME: triethylene glycol methyl ether; TEGDME: triethylene glycol dimethyl ether; EGEE: ethylene glycol ethyl ether; EGDEE: ethylene glycol diethyl ether; DEGEE: diethylene glycol ethyl ether; DEGDEE: diethylene glycol diethyl ether; TEGEE: triethylene glycol ethyl ether; EGBE: ethylene glycol butyl ether; DEGBE: diethylene glycol butyl ether, TEGBE: triethylene glycol butyl ether, EGPhE: ethylene glycol phenyl ether; PGME: propylene glycol methyl ether; MAA: methoxyacetic acid; EAA: ethoxyacetic acid;

EEAA: ethoxy-ethoxyacetic acid; BAA: 2-butoxyacetic acid; PhAA: phenoxy-acetic acid; 2-MPA: methoxy-proprionic acid.

Table 2. Characteristics of the study population (n = 169 mother-child pairs)

Characteristics (n = 169)	**n (%)**	**Mean ± SD**
Maternal characteristics		
Maternal age at conception (years)		30.8 ± 4.2
Fish consumption before pregnancy		
Once a week or less	114 (67.5)	
More than once a week	55 (32.5)	
Mother's education level		
Bachelor or less	60 (35.5)	
Higher education	109 (64.5)	
WAIS_QIV score		
< 84.8	39 (23.1)	
[84.8-93.0[	39 (23.1)	
[93.0-101.0[	45 (26.6)	
≥ 101.0	46 (27.2)	
Child characteristics		
Gender		
Male	83 (49.1)	
Female	86 (50.9)	
School level		
Nursery school	134 (79.3)	
Primary school	35 (20.7)	
Duration of videogame playing per week		
No videogames	67 (39.6)	
< 1h30	45 (26.6)	
≥ 1h30	57 (33.7)	
Regular extra-curricular sports		
No	52 (30.8)	
Yes	117 (69.2)	

Duration of TV watching per week	
< 2h30	56 (33.1)
[2h30-4h30]	52 (30.8)
> 4h30	61 (36.1)
Family characteristics at the 6-year follow-up	
Number of children at home	
1	7 (4.1)
2	96 (56.8)
3	54 (32.0)
≥ 4	12 (7.1)
Residence (at six years of age)	
Rural	91 (53.8)
Urban	78 (46.2)
HOME score	
< 44	35 (20.7)
[44-47[	51 (30.2)
[47-49[	34 (20.1)
≥ 49	49 (29.0)
Inhibition assessment of the child	
Psychologist	
n°1	86 (50.9)
n°2	83 (49.1)

Table 3. R-CPT90 test performance (n = 169).

	Proportion of correct responses (%)				
	Min	**Q1**	**Median**	**Q3**	**Max**
Target stimuli (n = 177) - *Compliance*	37.9	67.8	77.4	85.3	98.3
Non-target stimuli (n =20) - *Response inhibition*	5.0	40.0	50.0	65.0	95.0
***Response inhibition corrected for compliance*[1]**	4.8	29.8	37.9	47.9	76.4

[1]% of correct responses to non-target stimuli multiplied by % of correct responses to target stimuli.

Table 4. Detection frequencies and concentrations of urinary GE metabolites (n = 169).

Urinary metabolites	Detected samples	Frequency of detection	Concentration (*mg/L*)				
			Min.	Q1	Median	Q3	Max.
MAA	161	95.3%	< LOD	0.029	0.058	0.100	0.489
EAA	155	91.7%	< LOD	0.009	0.016	0.025	0.169
EEAA	153	90.5%	< LOD	0.011	0.028	0.082	8.463
BAA	167	98.8%	< LOD	0.021	0.039	0.072	0.284
PhAA	168	94.4%	< LOD	0.176	0.388	1.089	91.908
MPA	161	95.3%	< LOD	0.011	0.018	0.030	0.883

MAA: methoxyacetic acid; EAA: ethoxyacetic acid; EEAA: ethoxy-ethoxyacetic acid; BAA: 2-butoxyacetic acid; PhAA: phenoxy-acetic acid; 2-MPA: methoxy-proprionic acid; Q1: first quartile; Q3: third quartile. The limit of detection (LOD) was 0.003 mg/L for all metabolites.

Table 5. Association between the response inhibition score of children and prenatal urinary concentrations of GE metabolites (n = 169).

	Corrected response inhibition score			
Concentration (mg/L)	**$\beta_{adjusted}$**	**95% CI**	**p-trend[1]**	**Adjustment**
MAA (methoxyacetic acid)				**(a)**
< 0.029	Reference	-	0.45	
[0.029-0.058[	0.028	[-0.033; 0.088]		
[0.058-0.100[	-0.011	[-0.071; 0.049]		
≥ 0.100	0.039	[-0.022; 0.099]		
EAA (ethoxyacetic acid)				**(b)**
< 0.009	Reference	-	**0.03**	
[0.009-0.016[	-0.019	[-0.075 ; 0.038]		
[0.016-0.026[	-0.064	[-0.121 ; -0.007]		
≥ 0.026	-0.055	[-0.117 ; 0.006]		
EEAA (ethoxy-ethoxyacetic acid)				**(c)**
< 0.011	Reference	-	0.66	
[0.011-0.029[	0.017	[-0.043; 0.077]		
[0.029-0.082[	0.023	[-0.038; 0.085]		
≥ 0.082	0.013	[-0.049; 0.075]		
BAA (butoxyacetic acid)				**(d)**
< 0.021	Reference	-	0.69	
[0.021-0.039[	-0.036	[-0.094; 0.021]		
[0.039-0.072[	-0.024	[-0.083; 0.035]		
≥ 0.072	-0.017	[-0.076; 0.042]		
PhAA (phenoxyacetic acid)				**(e)**
< 0.176	Reference	-	0.84	
[0.176-0.388[	-0.011	[-0.073; 0.051]		
[0.388-1.090[	0.001	[-0.059; 0.061]		
≥ 1.090	-0.011	[-0.072; 0.050]		

MPA (methoxypropionic acid)			**(f)**
< 0.011	Reference	-	0.51
[0.011-0.018[	0.007	[-0.053; 0.068]	
[0.018-0.030[	-0.003	[-0.067; 0.061]	
≥ 0.030	-0.019	[-0.084; 0.045]	

[1]P-trend obtained with quartiles of concentrations recoded into numerical values.

(a) Adjusted for creatinine concentration, storage time between sample recovery and analysis, gestational age of mothers at inclusion, transport time at room temperature, sampling period, and duration of videogame playing.

(b) Adjusted for creatinine concentration, storage time between sample recovery and analysis, gestational age of mothers at inclusion, transport time at room temperature, sampling period, gender, place of residence, regular extra-curricular sports, and HOME score.

(c) Adjusted for creatinine concentration, storage time between sample recovery and analysis, gestational age of mothers at inclusion, transport time at room temperature, sampling period, duration of TV watching, and HOME score.

(d) Adjusted for creatinine concentration, storage time between sample recovery and analysis, gestational age of mothers at inclusion, transport time at room temperature, sampling period, place of residence, duration of videogame playing, and mother's fish consumption.

(e) Adjusted for creatinine concentration, storage time between sample recovery and analysis, gestational age of mothers at inclusion, transport time at room temperature, sampling period, and HOME score.

(f) Adjusted for creatinine concentration, storage time between sample recovery and analysis, gestational age of mothers at inclusion, transport time at room temperature, and sampling period.

Response inhibition is a cognitive control ability critical for everyday life

Urinary glycol ethers metabolites are ubiquitously present in prenatal samples

Response inhibition of six-year-olds decreased when ethoxyacetic acid level increased

No association was observed for the five other glycol ethers metabolites of interest

Knowledge on the possible exposure sources to glycol ethers is limited

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: