THÈSE DE DOCTORAT DE

L'UNIVERSITÉ DE RENNES 1
COMUE UNIVERSITÉ BRETAGNE LOIRE

ÉCOLE DOCTORALE N° 605
Biologie Santé
Spécialité : *Épidémiologie, Analyse de Risque, Recherche Clinique*

Par

Anne-Claire BINTER

Effets de l'exposition prénatale aux neurotoxiques sur le fonctionnement du cerveau de l'enfant évalué par imagerie cérébrale

Effects of prenatal exposure to neurotoxicants on the child's brain function evaluated by cerebral imaging

Thèse présentée et soutenue à Rennes, le 4 Décembre 2019
Unité de recherche : INSERM U1085 - Institut de recherche en santé, environnement et travail (Irset)

Rapporteurs avant soutenance :

	Isabelle Momas,	Professeur des universités | Université Paris Descartes
	Maryse Bouchard,	Professeure agrégée | Université de Montréal

Composition du jury :

Président :	Cyril Pernet,	Senior academic fellow | University of Edinburgh
Examinateurs :	Isabelle Momas,	Professeur des universités | Université Paris Descartes
	Maryse Bouchard,	Professeure agrégée | Université de Montréal
	Marion Mortamais,	Chercheur | Inserm UMR 1061
Dir. de thèse :	Cécile Chevrier,	Directrice de recherche | Inserm UMR 1085
Co-dir. de thèse :	Fabienne Pelé,	Docteur en Médecine | Inserm UMR 1085
	Elise Bannier,	Ingénieur de recherche hospitalier | CHU Rennes

Cette thèse a été financée par la Fondation de France et préparée dans le cadre du Réseau doctoral en santé publique animé par l'École des Hautes Études en Santé Publique (EHESP).

ACKNOWLEDGEMENT

Avant de commencer, je voudrais remercier toutes les personnes qui m'ont accompagnée, encouragée et soutenue pendant ces trois années.

En premier lieu mes directrices, Fabienne Pelé, Cécile Chevrier et Elise Bannier, pour votre patience et vos conseils avisés tout au long du parcours tortueux de la thèse, d'autant plus à la croisée des chemins disciplinaires !
Fabienne, pour ta confiance et la liberté que tu m'as laissée pour m'approprier ton projet, qui a pu devenir le mien.
Cécile, pour la transmission scientifique, tes encouragements et ta disponibilité.
Elise, pour la découverte d'un autre monde et d'une autre langue qu'est celle de l'imagerie, qui m'a permis de penser autrement mon travail.

Je remercie mes rapporteurs, Isabelle Momas et Maryse Bouchard, pour le temps consacré à la lecture critique de ce travail, ainsi que Cyril Pernet et Marion Mortamais, pour leur participation au jury de thèse.
Je remercie également les membres de mon comité de thèse, Dave Saint-Amour, Alexis Elbaz et Pascal Benquet pour leurs conseils et retours constructifs pendant ces trois années.
Je souhaite remercier les financeurs (Fondation de France, CHU de Rennes, EHESP et Université Bretagne-Loire), ainsi que l'équipe et les familles de PELAGIE sans qui ce travail n'aurait pas vu le jour.
Merci également aux collaborateurs du projet PERINE, Gregory Simon et Dave Saint-Amour pour leur expertise neuropsychologique et à l'équipe Empenn de l'Irisa, Christian Barillot et Julie Coloigner pour l'analyse IRM.

Je remercie Bernard Jegou, pour son accueil au sein de l'Institut de Recherche en Santé, Environnement et Travail (IRSET).
Un très grand merci aux membres de l'équipe 9, Luc Multigner, Sylvaine Cordier, Ronan Garlantézec, Nathalie Costet, Christine Monfort, Rémi Béranger, Bénédicte Jacquemin, Florence Rouget, pour leur bienveillance et leur bonne humeur, c'est un plaisir de travailler dans ces conditions.
Un grand merci également aux doctorants, post-docs et M2 que j'ai côtoyés pendant ces 3 ans et aux belles amitiés qui sont nées dans la galère de la thèse : Noriane, Zohra, Jade, vous en viendrez aussi à bout ! Courage !
Enfin, merci à celles et ceux qui ont relu ce manuscrit avant la date fatidique de l'impression !

Je remercie le réseau doctoral de l'EHESP, dont les formations m'ont fait découvrir la diversité de la recherche en santé publique et pour le soutien financier qui m'a permis de m'évader en 3e année à l'autre bout du monde !
Many thanks at CERCH, Brenda Eskenazi for welcoming me in her team, Ana Maria

Mora and Sharon Sagiv for our discussions, their relevant advices and sharing their vision of environmental epidemiological research, and to all students, post-doctoral fellows and team members for their warm hospitality.

Enfin, merci aux amis et à ma famille pour les bons moments passés à Rennes, Nantes et ailleurs. Merci en particulier à mes frères, Florian, Pierre-Antoine, pour votre soutien si précieux.
A ma mère-veilleuse maman, mon indéfectible pilier.
Et à Nicolas, pour ta compréhension, tes encouragements et ton amour.

A mon grand-père,
A mes parents,

Publications

Binter A-C., Bannier E., Saint-Amour D., Simon G., Ferré J-C., Barillot C., Monfort C., Cordier S., Chevrier C., Pelé F., Prenatal exposure to glycol ethers and motor inhibition function evaluated by functional MRI at the age of 10 to 12 years in the PELAGIE mother-child cohort. *Environ Int. sep 2019;133:105163.*

Binter A-C., Bannier E., Saint-Amour D., Simon G., Barillot C., Monfort C., Cordier S., Pelé F.*, Chevrier C.*, Exposure of pregnant women to organophosphate insecticides and motor inhibition at the age of 10 to 12 years evaluated by fMRI (PELAGIE mother-child cohort). [In revision in *Environmental Research.*]

Binter A-C., Simon G., Chevrier C., Pelé F., Bannier E., Motor inhibition BOLD responses and NEPSY scores at the age of 10 to 12 years (PELAGIE mother-child cohort). [In preparation.]

Oral communications

Binter A-C., Bannier E., Saint-Amour D., Simon G., Barillot C., Cordier S., Chevrier C., Pelé F., Exposure of pregnant women to organophosphate insecticides and motor inhibition evaluated by functional MRI at the age of 10 to 12 years (PELAGIE mother-child cohort). *International Society of Environmental Epidemiology (ISEE)*. August 2019. Utrecht.

Binter A-C., Bannier E., Saint-Amour D., Simon G., Ferré J-C., Barillot C., Viel J-F., Cordier S., Chevrier C., Pelé F., Prenatal solvent exposure association with motor inhibition functional MRI at the age of 10-12 in the PELAGIE mother-child cohort. *International Society of Environmental Epidemiology (ISEE)*. September 2017. Sydney.

Binter A-C., Bannier E., Cordier S., Viel J-F., Chevrier C., Pelé F., Prenatal solvent exposure and motor inhibition function at the age of 10 – 12 in the PELAGIE mother-child cohort. *International Society of Environmental Epidemiology (ISEE)*. September 2017. Sydney.

Binter A-C., Bannier E., Saint-Amour D., Simon G., Barillot C., Monfort C., Cordier S., Chevrier C.*, Pelé F.*, Exposure of pregnant women to organophosphate insecticides and motor inhibition evaluated by functional MRI at the age of 10 to 12 years (PELAGIE mother-child cohort). *Scientific Meetings of the Public Health Doctoral Network*. March 2019. Paris.

Binter A-C., Bannier E., Saint-Amour D., Simon G., Barillot C., Monfort C., Cordier

S., Chevrier C.*, Pelé F.*, Exposure of pregnant women to organophosphate insecticides and motor inhibition evaluated by functional MRI at the age of 10 to 12 years (PELAGIE mother-child cohort). *Printemps de la recherche à l'Irset*. March 2019. Rennes.

Poster presentations

Binter A-C., Bannier E., Saint-Amour D., Simon G., Ferré J-C., Barillot C., Cordier S., Chevrier C., Pelé F., Prenatal exposure to glycol ethers and motor inhibition function evaluated by functional MRI at the age of 10 to 12 years in the PELAGIE mother-child cohort. *European doctoral college on Environment and Health (EDCEH)*. June 2018. Rennes.

Binter A-C., Bannier E., Saint-Amour D., Simon G., Ferré J-C., Barillot C., Cordier S., Chevrier C., Pelé F., Prenatal exposure to glycol ethers and motor inhibition function evaluated by functional MRI at the age of 10 to 12 years in the PELAGIE mother-child cohort. *Scientific Meetings of the Public Health Doctoral Network*. March 2018. Bordeaux.

Binter A-C., Pelé F., Chevrier C., Statistical standardization method for correcting the influence of sampling conditions: application on urinary biomarkers of exposure to organophosphate pesticides. *Journées scientifiques de l'école doctorale Biologie-Santé de l'UBL*. December 2018. Rennes.

Research stay abroad

Binter A-C., Sagiv S., Mora A., ..., Eskenazi B., Prenatal and childhood exposure to DDT and DDE and functional neuroimaging in adolescents from the CHAMACOS cohort. [In preparation.]

Mediation

Rennes semi-finals, Ma thèse en 180 secondes. March 2019.

Other contributions

Warembourg C.*, **Binter A-C.*** *et al.*, Prenatal exposure to glycol ethers and sex steroid hormones at birth. *Environ Int. apr 2018;113:66-73.*

Beranger R.*, Hardy E.*, **Binter A-C.** *et al.*, Multiple pesticides in mothers' hair samples and children's measurements at birth: results from the French national birth cohort (ELFE). *International Journal of Hygiene and Environmental Health. In press.*

SUMMARY

Le concept des origines développementales de la santé et des maladies (developmental origin of health and diseases, DOHAD) vise à comprendre comment l'environnement pendant le début de la vie (généralement défini comme les mille premiers jours de vie, de la conception aux deux ans de l'enfant) programme le fonctionnement des organes et des systèmes pour la vie entière et influence l'état de santé. L'environnement sera entendu ici comme la combinaison complexe des facteurs physiques et chimiques, des conditions et circonstances de vie qui entourent chaque individu. Le développement de la structure et du fonctionnement du cerveau est connu pour être une période cruciale de sensibilité et de vulnérabilité. Un nombre important d'études scientifiques antérieures et actuelles étudient le lien entre l'environnement et le neurodéveloppement ainsi que le vieillissement cognitif. De plus, l'incidence des pathologies neurologiques affectant à la fois l'enfant (e.g. déficit de l'attention et hyperactivité, troubles du spectre autistique) et l'adulte (e.g. maladie d'Alzheimer, accidents vasculaires cérébraux) a augmenté dans les dernières décennies. D'autre part, l'usage croissant de produits chimiques confirme la nécessité de poursuivre les investigations et d'améliorer notre compréhension de phénomènes de santé aux mécanismes complexes.

Cette thèse s'appuie sur la cohorte mère-enfant PELAGIE (Perturbateurs Endocriniens : Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance). Son schéma d'étude offre la possibilité d'investiguer les effets de l'exposition prénatale aux composés neurotoxiques à une échelle populationnelle. Les échantillons biologiques collectés en début de grossesse sont utilisés pour étudier deux classes de produits chimiques : les éthers de glycol et les insecticides organophosphorés.

Cette recherche apporte des connaissances à propos des effets sur la santé humaine de l'exposition *in-utéro* à des contaminants retrouvés fréquemment dans l'environnement de la population générale. De plus, elle approfondit l'examen de possibles modes d'actions neuronaux grâce à l'imagerie par résonance magnétique fonctionnelle (functional magnetic resonance imaging, fMRI), dont l'utilisation dans le contexte de l'épidémiologie environnementale est innovante.

Ce travail présente deux objectifs principaux : le premier est d'investiguer si des effets similaires à ceux décrits dans la littérature peuvent être observés sur l'inhibition motrice d'enfants de 10-12 ans recrutés en population générale dans une cohorte mère-enfant prospective (la cohorte PELAGIE). Le second objectif est d'utiliser une technique d'imagerie cérébrale, ici l'IRM fonctionnelle (IRMf), pour étudier les possibles mécanismes neuraux responsables des altérations cérébrales suggérées par le corpus des études publiées.

Trois études ont été conduites pour répondre à ces objectifs :

1. Réponses BOLD pendant une tâche d'inhibition motrice chez des enfants de 10-12 ans et associations avec les scores d'attention et d'inhibition de tests neuropsychologiques.

2. Effets de l'exposition prénatale aux éthers de glycol sur l'inhibition motrice évaluée par IRMf.

3. Effets de l'exposition prénatale aux insecticides organophosphorés sur l'inhibition motrice évaluée par IRMf.

L'objectif de la première étude est de décrire les activations cérébrales pendant une tâche de Go/No-Go d'enfants de 10 à 12 ans recrutés en population générale et d'évaluer les corrélations entre ces activations et les scores de tests neuropsychologiques d'attention et d'inhibition.
Une tâche visuelle de Go/No-Go a été présentée aux enfants de la cohorte PELAGIE et cette étude est restreinte aux droitiers (n = 87). Les scores d'attention sont issus des sous-tests d'attention visuelle et attention auditive de la NEPSY. Les scores d'inhibition sont construits à partir des performances à la tâche de Go/No-Go. La tâche de Go/No-Go a été réalisée pendant une séquence d'IRM fonctionnelle. Cinq contrastes ont été construits à partir des images : "Go réussis", "No-Go réussis", "No-Go échoués", "No-Go réussis vs Go réussis" et "No-Go vs Go réussis". Les estimateurs extraits du modèle général linéaire ont permis d'obtenir les clusters activés pour chaque contraste. Les activations pour chaque région d'intérêt ont été moyennés. Les corrélations entre les activations et les scores d'attention et d'inhibition ont été estimées par des coefficients de Spearman entre les estimateurs des régions d'intérêt et les scores.
A partir des contrastes spécifiques à l'inhibition ("No-Go réussis vs Go réussis" et "No-Go vs Go réussis"), nous avons observé des activations cérébrales dans les régions frontales (bilatéralement) dont le cortex inférieur frontal latéral, le cortex cingulaire antérieur et l'aire motrice supplémentaire. Les scores neuropsychologiques étaient faiblement corrélés aux activations cérébrales.

Les femmes enceintes sont fréquemment exposées aux solvants organiques, tels que les éthers de glycol. Plusieurs études suggèrent une potentielle toxicité de l'exposition prénatale aux éthers de glycol sur le cerveau en développement mais les possibles mécanismes neuronaux restent mal définis. Ainsi, l'objectif de la deuxième étudier est d'évaluer l'association entre les niveaux d'éthers de glycol dans les urines de femmes en début de grossesse et la fonction inhibitrice de leurs enfants âgés de 10 à 12 ans mesurées par des tests comportementaux et par imagerie cérébrale.
L'exposition aux éthers de glycol est estimée en mesurant six métabolites urinaires de 73 femmes enceintes (avant 19 semaines de gestation) recrutées dans la cohorte mère-enfant PELAGIE (France). Les niveaux urinaires sont classés en faibles,

modérés, élevés. Les enfants ont passé un examen d'imagerie par résonance magnétique fonctionnelle (IRMf) durant lequel la fonction d'inhibition motrice est évaluée par une tâche de Go/No-Go. Les analyses statistiques sont réalisées en utilisant des modèles de régression linéaire pour les performances à la tâche et des modèles linéaires généralisés à effets mixtes pour les activations cérébrales, corrigés pour tests multiples au niveau des clusters (correction FWER). Les facteurs de confusion sont pris en compte par restriction à l'inclusion ou par ajustement des modèles.
Les concentrations urinaires d'acide butoxyacétique (BAA) les plus élevées étaient associées à de plus faibles performances chez l'enfant (β = - 1.1; intervalle de confiance à 95% (IC95%) : - 1.9, - 0.2 for high vs low). Nous avons également observé une tendance à de plus faibles performances en lien avec l'acide éthoxyacétique (EAA) (β = - 0.3; IC95% : - 0.7, 0.01). En considérant la consigne d'inhibition, l'activité dans les régions occipitales augmente en association avec des niveaux modérés d'EAA (cuneus gauche) et d'acide méthoxyacétique (MAA) (precuneus droit). Lorsque les enfants réussissaient à inhiber, les niveaux élevés d'acide ethoxyethoxyacétique (EEAA) et modérés d'acide phénoxyacétique (PhAA) étaient associés avec des activités différentielles (diminution et augmentation, respectivement) dans le cortex frontal, une région cérébrale impliquée dans le réseau de l'inhibition.
Les niveaux urinaires prénataux de deux métabolites d'éthers de glycol ont été associés à de plus faibles performances à la tâche de Go/No-Go. Des activations différentielles ont été observées dans les régions du cerveau impliquées dans l'inhibition en cas d'inhibition réussie mais pas en considérant la demande d'inhibition. Il n'y a cependant pas de cohérence entre les indicateurs de performances et les résultats sur les activations cérébrales. D'autres études sont nécessaires en raison du caractère ubiquitaire de l'exposition aux éthers de glycol.

Enfin, nous cherchons à investiguer les effets de l'exposition prénatale aux OP sur le contrôle inhibiteur d'enfants âgés de 10 à 12 ans évalué par une tâche d'inhibition motrice pendant une séquence d'IRM fonctionnelle.
Les insecticides organophosphorés (OP) sont très utilisés, notamment en agriculture. Plusieurs études épidémiologiques suggèrent une potentielle toxicité neurodéveloppementale chez l'enfant après exposition à des niveaux faibles, correspondant à ceux de la population générale, mais les mécanismes d'action restent peu compris.
Quatre-vingt quinze enfants de la cohorte PELAGIE (Bretagne-France, à partir de 2002) ont réalisé une tâche de Go/No-Go pendant une séquence d'IRM fonctionnelle avec la consigne suivante : appuyer le plus vite possible sur un bouton lorsqu'un smiley vert apparaissait, mais ne pas répondre aux smileys rouges (300 smileys successifs pendant 10 minutes). Les performances à la tâche ont été évaluées par le temps de réaction moyen, le taux de commission et un score composite de perfor-

mance. Les activations cérébrales sur l'ensemble du cerveau ont été estimées en modélisant la réponse hémodynamique liée à la consigne d'inhibition et la réussite à supprimer le geste. L'exposition aux OP était évaluée en mesurant les concentrations de six métabolites (dialkylphosphate, DAP) dans les urines maternelles en début de grossesse (avant 19 semaines de gestation). Les concentrations ont été sommées pour obtenir les niveaux totaux de diethylphosphate (DE), dimethyphosphate (DM) et l'ensemble des métabolites non-spécifiques (DAP). Les niveaux ont été standardisés pour homogénéiser les conditions d'échantillonnage et catégorisés en 3 classes d'exposition : faible (référence), modérée et élevée. Les facteurs de confusion potentiels ont été pris en compte par restriction ou ajustement.
Les niveaux modérés de DAP étaient associés avec une diminution du taux de commission ($p = 0.04$), sans que le score de performance augmente. On observait une tendance à la diminution du score de performance avec des niveaux modérés de DE ($p = 0.06$).
L'augmentation des niveaux urinaires de DM et DE était associée avec une diminution des activités cérébrales dans les régions frontales inférieures gauches et supérieures (bilatéralement) pendant l'inhibition réussie. Il n'y avait pas de différence d'activations pendant la demande d'inhibition.
Nous n'avons pas trouvé d'altérations majeures des scores neuropsychologiques mais nos résultats suggèrent des diminutions de l'activité cérébrale dans des régions liées à l'inhibition après exposition prénatale aux OP. Ces résultats restent à confirmer par des études avec d'autres populations.

Ce travail présente plusieurs limites, notamment méthodologiques. Ainsi l'utilisation de biomarqueurs urinaires d'exposition, les choix de la tâche de Go/No-Go ainsi que la modélisation statistique des activations cérébrales sont discutés dans la quatrième partie de la thèse.
Cette revue critique des résultats permet d'ouvrir à différentes implications de cette recherche pour la santé publique. Si les résultats obtenus suggèrent une association entre l'exposition prénatale aux contaminants environnementaux et le contrôle inhibiteur, les associations statistiques ne sont pas une preuve de causalité. L'identification des mécanismes biologiques de neurotoxicité est un moyen d'établir la causalité. Cependant, la diversité des mécanismes possibles et les limites spécifiques des expérimentales (qu'elles soient réalisées in-vitro ou sur modèles animal) compliquent cet objectif. Ainsi, pour s'approcher de la causalité en épidémiologie il serait pertinent de répliquer ces résultats, de promouvoir les recherches à long-terme et d'intégrer les facteurs environnementaux à la fois chimique et sociaux, dans les recherches futures.

CONTENTS

SIGNIFICANCE

I SCIENTIFIC CONTEXT

1 The brain: anatomy and function

1.1 Role and structures of the nervous system 3
1.2 Anatomy and functions of the brain 3
1.3 The executive functions 9
1.4 Visualizing brain with cerebral imaging 12
1.5 Embryogenesis of the brain and neurodevelopment 18

2 Neurodevelopmental alterations

2.1 Generalities about behavioral and cognitive disorders 23
2.2 The brain and environment 24

3 Glycol ethers: neurotoxicant solvents?

3.1 Definition and properties 31
3.2 Uses in France 31
3.3 Regulations in France and UE 33
3.4 Toxicology 35
3.5 Developmental neurotoxicant effects 37

4 Organophosphates, neurotoxicant insecticides

4.1 Definition and properties 43
4.2 Uses and exposure in France 43
4.3 Toxicology 45
4.4 Neurotoxicant effects 47

5 Objectives

II METHODOLOGIES

1 The PELAGIE cohort

1.1 Study protocols 61
1.2 The PERINE study 61

2 Prenatal exposure assessment

2.1 Use of biomarkers of exposure 65
2.2 Exposure to glycol ethers 65
2.3 Exposure to organophosphate insecticides 66

3 Evaluation of child's motor inhibition function

3.1 Paradigm of the Go/No-Go task 67
3.2 Neuropsychological assessment: performance indicators 68
3.3 Brain activities related to motor inhibition: contrast(s) of interest 68

4 Statistical analyses

4.1 Homogenizing sampling conditions of exposure assessment 71
4.2 Taking into account confounding factors 72

4.3 Statistical modeling 73

III RESULTS

1 Motor inhibition BOLD responses and NEPSY scores

1.1 Résumé 77
1.2 Motor inhibition BOLD responses and NEPSY scores 77

2 Glycol ethers and motor inhibition

2.1 Résumé 99
2.2 Prenatal exposure to glycol ethers and motor inhibition 100

3 Organophosphate and motor inhibition

3.1 Résumé 115
3.2 Prenatal exposure to OP and motor inhibition 116

IV CONCLUSION

1 Review

1.1 Synthesis of the main findings 159
1.2 Methodological considerations 161

2 Challenges and Perspectives

2.1 Implications in public health and the gap to causal proof 167
2.2 Issues of interdisciplinary researches 172
2.3 Perspectives of research 174

BIBLIOGRAPHY

References 177

APPENDIX

Physical principles of MRI

Diagnostic of ADHD

Phytopharmaceutical use of organophosphates in France in 2019

TABLES

3.1 Regulations of GE compounds in France 34
3.2 Alkoxycarboxylic acids produced by metabolization of GE 36
3.3 Studies on solvents and neurodevelopment 39

4.1 Epidemiological studies on organophosphate (OP) and neurodevelopment 51

FIGURES

1.1 Anatomy of the adult brain 4
1.2 Lobes of the human brain 5
1.3 Structure of a neuron 6
1.4 Schema of an electrochemical synapse 8
1.5 Magnetic moment 13
1.6 Brain regions involved in inhibition 17
1.7 Cross-sectional view of the formation of the neural groove 19
1.8 Windows of development of the human brain 21

3.1 Molecular formulas of some glycol ethers (GE) 32
3.2 Metabolism of glycol ethers 36

4.1 Molecular formulas of OP insecticides 44

1.1 Flowchart of the PELAGIE cohort 62

3.1 Paradigm of the Go/No-Go task 67
3.2 Image analysis using SPM 69

4.1 Statistical standardization of OP metabolites 72
4.2 Continuous random field 73

2.1 Modes of actions of environmental contaminants 168

1PG2EE 1-propylene glycol 2-ethylether
1PG2ME 1-propylene glycol 2-methylether
1PG2MEA 1-propylene glycol 2-methylether acetate
2-BEAA 2-butoxyethoxypropionic acid
2-EPA 2-ethoxypropionic acid
2-MPA 2-methoxypropionic acid
2-PhPA 2-phenoxypropionic acid
ACh Acetylcholine
AChE acetylcholinesterase
ADHD attention-deficit/hyperactivity-disorder
ATP adenosine triphosphate
BAA butoxyacetic acid
BBB blood brain barrier
BEAA butoxyethoxyacetic acid
BOLD Blood oxygenation level dependent
CCCEH Columbia Center for Children's Environmental Health
CHAMACOS Center for the Health Assessment of Mothers and Children of Salinas
CMR carcinogenic, mutagenic and reprotoxic
CNS central nervous system
CPT Conners' Continuous Performance Test
CSF cerebrospinal fluid
CYP450 cytochromes P450
DAP dialkylphosphate
DDT dichlorodiphenyltrichloroethane
DE diethylphosphate
DEDTP diethyldithiophosphate
DEGDEE diethylene glycol diethylether
DEGDME diethylene glycol dimethylether
DEGEE diethylene glycol ethylether
DEGME diethylene glycol methylether
DETP diethylthiophosphate
DEGBE diethylene glycol monobutylether
DEP diethylphosphate
dHb deoxygenated hemoglobin
DM dimethylphosphate
DMDTP dimethyldithiophosphate
DMTP dimethylthiophosphate
DMP dimethylphosphate
DPGnBE dipropylene glycol n-butylether
DPGME dipropylene glycol methylether
DTI diffusion tensor imaging
EGBE ethylene glycol butylether
EGDEE ethylene glycol diethylether
EGDME ethylene glycol dimethylether
EGEE ethylene glycol ethylether
EGEEA ethylene glycol ethylether acetate
EGME ethylene glycol methylether
EGMEA ethylene glycol methylether acetate
EGPhE ethylene glycol phenylether
EGiPE ethylene glycol isopropylether
EGnPE ethylene glycol n-propylether
EAA ethoxyacetic acid
ECs endothelial cells
EEAA ethoxyethoxyacetic acid
FDR false discovery rate
FID free-induction decay
fMRI functional MRI
fNIRS functional near infra-red spectroscopy
FWER family-wise error rate
GABA gamma-aminobutyric acid
GC-MS gas chromatography coupled to mass-spectrometry

GE glycol ethers

GHS Globally Harmonized System of classification and labelling of chemicals

GIS Geographic information system

GLM generalized linear model

GM gray matter

HCB hexachlorobenzene

HOME Study Health Outcomes and Measures of the Environment Study

HR haemodynamic response

IQ Intelligence Quotient

JEM Job-exposure matrix

LC-SMS liquid chromatography-electrospray ionization tandem mass spectrometry

LD50 median lethal dose

LOD limit of detection

MAA methoxyacetic acid

MEAA methoxyethoxyacetic acid

MR Magnetic Resonance

MRI Magnetic Resonance Imaging

NEPSY developmental NEuroPSYchological assessment

NHANES National Health and Nutrition Examination Survey

NMDA N-methyl-D-aspartate

NMR nuclear magnetic resonance

oHb oxygenated hemoglobin

OP organophosphate

PAA propoxyacetic acid

PBDEs polybrominated diphenyl ethers

PCBs polychlorinated biphenyls

PELAGIE Perturbateurs Endocriniens : Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance

PET Positron Emission Tomography

PGBE propylene glycol butylether

PGEE propylene glycol ethylether

PGME propylene glycol methylether

PGPE propylene glycol propylether

PhAA phenoxyacetic acid

PK/PD pharmacokinetic/pharmacodynamic

PNS peripheral nervous system

PON1 paraoxonase 1

ppm parts-per-million

PUR Pesticide-use report

REACH Registration, Evaluation, Authorization and restriction of CHemicals

ROI regions of interest

SMA supplementary motor area

SPE solid-phase extraction

SPM Statistic Parametric Mapping

SUMER Surveillance médicale des expositions des salariés aux risques professionnels

TE echo time

TEGBE triethylene glycol butylether

TEGDME triethylene glycol dimethylether

TEGEE triethylene glycol ethylether

TEGME triethylene glycol methylether

TR repetition time

VBM Voxel-Based Morphometry

VOC volatile organic compounds

WG weeks of gestation

WISC Wechsler Intelligence Scale for Children

WM white matter

SIGNIFICANCE

The developmental origin of health and diseases (DOHAD) is a concept whose purpose is to understand how the environment during the beginning of life (commonly the first thousand days from conception to age two years) programs the function of organs and systems for the entire life and influences health. The environment considered here as the complex combination of physical, chemical factors, the circumstances, or conditions by which one is surrounded.
Development is known to be a key period of vulnerability and sensitivity to environments for the brain structure and function. A significant number of scientific studies have and are still investigating the link between environment and neurodevelopment and cognitive aging.
On the one hand, incidence and interest in neurological pathologies affecting both children (e.g. attention deficit-hyperactivity disorders, autism spectrum disorders), and adults (e.g. stroke, Alzheimer's disease) have grown in the last decades. On the other hand, the increasing use of chemicals supports the need to follow the investigations on the possible effects of these compounds on human health and improve our understanding of complex mechanistic phenomena.

This dissertation builds upon the PELAGIE (Perturbateurs Endocriniens : Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance) mother-child cohort, which offers the opportunity to investigate effects of prenatal exposure to neurotoxicants at a population scale. Biological samples collected during pregnancy are used to study two classes of chemicals: organophosphate insecticides and glycol ethers.
This research provides information about human health effects of prenatal exposure to contaminants commonly found in the environment of the general population. It offers a closer look of possible neural underpinnings through the use of functional magnetic resonance imaging (fMRI), which use in the context of epidemiological studies is innovative.

The first part of the dissertation lays the basis of the function and the development of the brain with a specific focus on inhibitory control, and presents general notions on the two chemical pollutants of interest. The second part describes the methodologies used for this work. The results are presented in a third part as scientific papers. The last part discusses the main findings and opens up for future research.

PART I

SCIENTIFIC CONTEXT

1.1. Role and structures of the nervous system

An organism communicates with the outside world and controls many mechanisms inside itself at the same time through the nervous system. The nervous system transmits signals to and from different parts of the organism. It detects environmental changes that affect the body through senses, processes the information, then responds to such events.

In vertebrates, the nervous system consists of the central nervous system (CNS) and the peripheral nervous system (PNS). The brain, safely contained within the skull, and the spinal cord in the vertebral canal form the central nervous system. The peripheral nervous system mainly consists of nerves, long fiber bundles, connecting the CNS to every other part of the body.

A distinction can also be made between voluntary and involuntary nervous system. However, these two parts are closely linked in the CNS, and they are usually more distinct in other parts of the body.
The somatic nervous system controls all the things that can be consciously influenced, such as voluntary movements.
Both autonomic and enteric nervous systems function involuntarily. They are constantly active, can react quickly to changes, regulating processes such as heartbeat and metabolic processes. Autonomic nervous system is subdivided into the sympathetic and the parasympathetic nervous systems. Roughly, they have opposite functions, sympathetic nervous system being activated in cases of emergencies to mobilize energy, while the parasympathetic nervous system is activated when organisms are in a relaxed state. Enteric nervous system controls the gastrointestinal system, autonomously regulates bowel motility in digestion[1].

1.2. Anatomy and functions of the brain

1.2.1. Divisions

There are three major anatomical divisions of the brain (cf. Figure 1.1): the hindbrain, the midbrain, and the forebrain[2].
The hindbrain extends from the spinal cord and is composed of the metencephalon and myelencephalon. The metencephalon contains the pons and cerebellum. The cerebellum structure is similar to the cerebral cortex, with two hemispheres and a highly folded cortex. It controls movement coordination, maintains balance and equilibrium.

1. Möller and Reif, *Atlas de poche d'anatomie radiologique*; Rohkamm and Grosshans, *Atlas de poche de neurologie*
2. Moussakova et al., *Anatomie et physiologie humaines*

The myelencephalon is composed of the medulla oblongata, responsible for controlling autonomic functions such as breathing, heart rate, and digestion.
The midbrain and the hindbrain together form the brainstem. It is involved in auditory and visual responses, motor function and basic vital functions.
The forebrain is the division of the brain responsible for receiving and processing sensory information, controlling motor function and thinking. There are two major divisions of forebrain: the diencephalon and the telencephalon.
The diencephalon contains structures such as the thalamus and hypothalamus that are responsible for motor control and relaying sensory information. The telencephalon contains the largest part of the brain, the cerebral hemispheres or cerebrum, accounting for two thirds of the total weight of the brain (around 1.4 kg in total). The cerebrum is associated with higher brain function such as thought and action. The cerebral hemispheres consist of an inner core of myelinated nerve fibers, the white matter (WM), and an outer cortex of gray matter (GM) with neuron cell bodies[1].

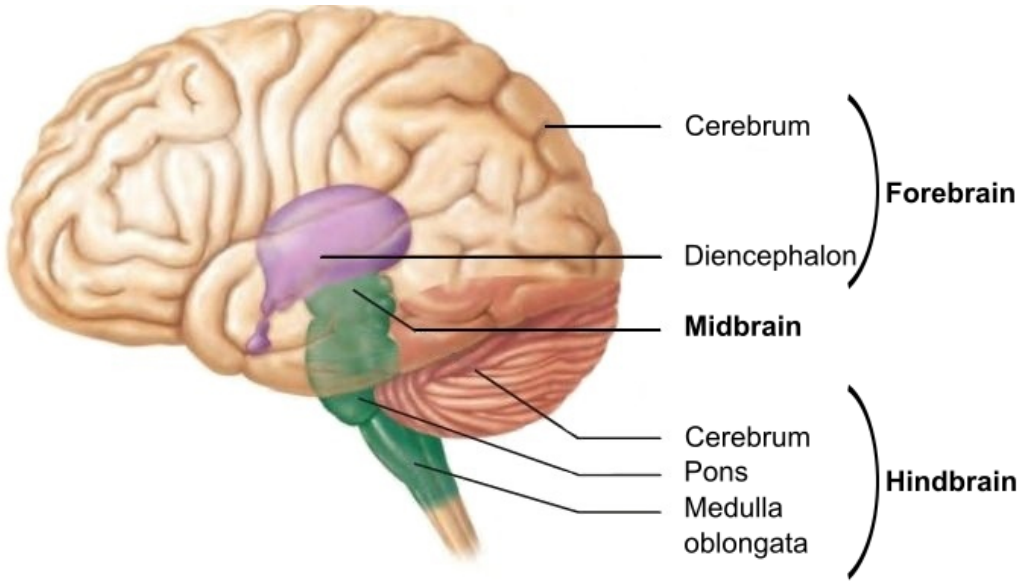


Figure 1.1 – Anatomy of the adult brain [Adapted from Moussakova et al., *Anatomie et physiologie humaines*].

The cerebral cortex is several millimeters thick and has a surface area of about 2,000 cm^2 because of an elaborate series of convolutions[3]. Nerve fibers in the WM connect functional areas of the cerebral cortex.
The GM of the cortex can be divided by major surface folds into four lobes (cf. Figure 1.2):

- The frontal lobe, associated with movement, speech, emotions, planning, and problem solving.

3. Encyclopaedia Britannica Publishers and Getty Images, *The Encyclopedia Britannica*

- The parietal lobe, associated with movement, orientation, and recognition of stimuli.
- The occipital lobe, associated with vision and color recognition, and visual processing.
- The temporal lobe, associated with perception and recognition of auditory stimuli, memory, and speech.

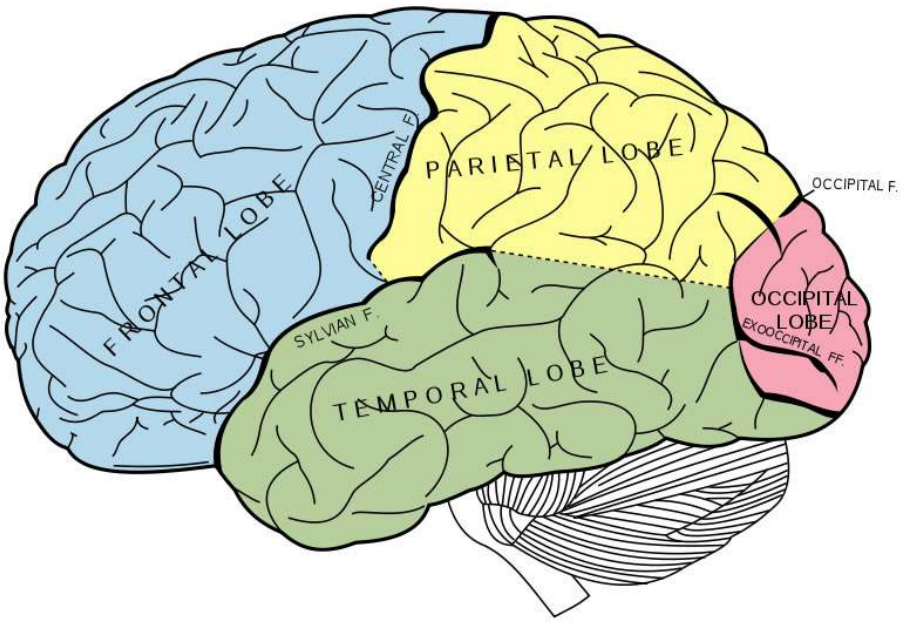


Figure 1.2 – Lobes of the human brain.

1.2.2. Neuronal and glial cells

At the cellular level, the human brain is composed of about a hundred billion nerve cells, also called neurons, and about an equal number of supporting elements or glial cells.
Each neuron has a cell body containing the nucleus, also called soma, and various extensions (cf. Figure 1.3). The dendrites receive signals and pass them on to the cell body. The signals are then sent via the axon, a long extension that can be up to a meter long.
Neurons send these signals in the form of electrochemical waves of action potentials and release neurotransmitter chemicals at synapse junctions. Most neurons send signals via their axons, although some of them are capable of dendrite-to-dendrite communication.

Glial cells (from the Greek "glue") are non-neuronal cells that supply nutrients to neurons, insulate neurons electrically, destroy pathogens and remove dead neurons,

and provide guidance cues directing the growing axons of neurons to their targets. There are three main types of glial cells: microglia, oligodendrocytes and astrocytes. Microglia cells are responsible for immune defense of the CNS by digesting cells debris and foreign bodies. Oligodendrocytes generate layers of myelin in the CNS, a fatty substance wrapped around axons providing electrical insulation that allows them to transmit signals rapidly and efficiently. Then, astrocytes provide a cellular link between the neuronal circuitry and blood vessels. This neurovascular coupling enables to relay signals that regulate blood flow in response to neuronal activity[4].

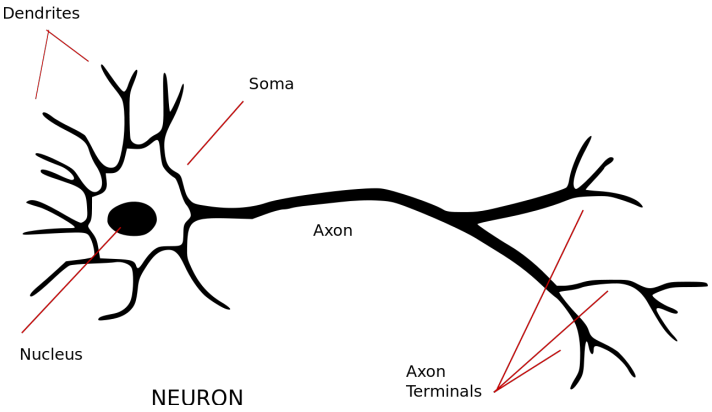


Figure 1.3 – Structure of a neuron.

1.2.3. Synapses and neurotransmitters

The majority of neurons is connected to thousands of other nerve cells, but some have relatively few connections with other neurons or organs. The total number of functional connections, or synapses, within the human brain is about a hundred trillion.
Functions of the brain are determined by the ability of neurons to transmit electrochemical signals to other cells, and their ability to respond appropriately to electrochemical signals received from other cells.
Synapses may be electrical or chemical. Electrical synapses make direct electrical connections between neurons, but chemical synapses are much more common, and much more diverse in function.

At a chemical synapse, the presynaptic cell contains neurotransmitter chemicals packed in vesicles. The presynaptic terminal is separated from the postsynaptic cell by the synaptic cleft. When an action potential arrives at the presynaptic terminal of the neuron, the neurotransmitter molecules are released through the presynaptic membrane and into the synaptic cleft (cf. Figure 1.4). In milliseconds, they travel across the synaptic cleft to the membrane of the postsynaptic cell, where they bind

4. Coumoul, *Toxicologie*; Gurden, “Astrocytes: can they be the missing stars linking neuronal activity to neurofunctional imaging signals?”

to receptors. The termination of neurotransmitter activity happens in several different ways. The molecules may diffuse out of the synaptic cleft (e.g. gamma-aminobutyric acid (GABA) molecules captured by glial cells), be taken back up into the presynaptic terminal via transporter molecules (e.g. dopamine or serotonin metabolisms), or be metabolized by enzymes in the synaptic cleft (e.g. Acetylcholine (ACh) degraded by acetylcholinesterase (AChE)).
Receptors are divided into two broad groups: chemically gated ion channels and second messenger systems. When a chemically gated ion channel is activated, it forms a passage that allows specific types of ions to flow across the membrane. Depending on the type of ion, the effect on the target cell may be excitatory or inhibitory. When a second messenger system is activated, it starts a cascade of molecular interactions inside the target cell, which may ultimately produce a wide variety of complex effects, such as increasing or decreasing the sensitivity of the cell to stimuli, or even altering gene transcription. According to a rule called Dale's principle, a neuron usually releases the same neurotransmitters at all of its synapses. However, because there are different types of receptors, it is possible for a neuron to have excitatory effects on one set of target cells, inhibitory effects on others, and complex modulatory effects on others[3].

The two neurotransmitters that are used in nearly every part of the brain are glutamate, which almost always exerts excitatory effects on target neurons, and GABA, which is usually inhibitory.
Other chemical neurotransmitters are used in more limited areas of the brain, often linked to particular functions. They can be classified based on chemical and molecular properties. The major classes of neurotransmitters include amino acids, such as glutamate and glycine; monoamines, such as dopamine and norepinephrine; peptides, such as somatostatin and opioids; and purines, such as adenosine triphosphate (ATP). ACh derives from the acetylation of choline, a vitamin-like nutrient. It is one of the major signal substances in the CNS, the transmitter in the cholinergic pathways. The cholinergic system is involved in many behavioral phenomena, such as memory and learning, audition, vision, aggressiveness and neurological syndromes[5].

The interconnexions by synapses between neurons form neural circuits that serve similar functions, for example sensory systems such as vision or hearing, or motor systems and are then grouped in neural systems. However, numerous circuits are less well defined and are responsible for the most complex brain functions. They are collectively referred to as associational systems.

Neurons and synapses have memorization abilities, the best-known form is a process called long-term potentiation, first discovered in glutamate using-synapses

5. Tata et al., "Cholinergic system dysfunction and neurodegenerative diseases".

acting on N-methyl-D-aspartate (NMDA) receptors. If several cells involved in the synapse are activated at approximately the same time, a channel opens that permits Calcium (Ca) to flow into the target cell. It initiates a second messenger cascade that leads to an increase in the number of glutamate receptors, enhancing the activity of the synapse. This change in strength can last at least for weeks. Other types of synaptic memory traces have been found, involving increases or decreases in synaptic strength, and lasting for variable periods. All these forms of synaptic modifiability give the capability for the nervous system to adapt itself to variations in the environment[6].

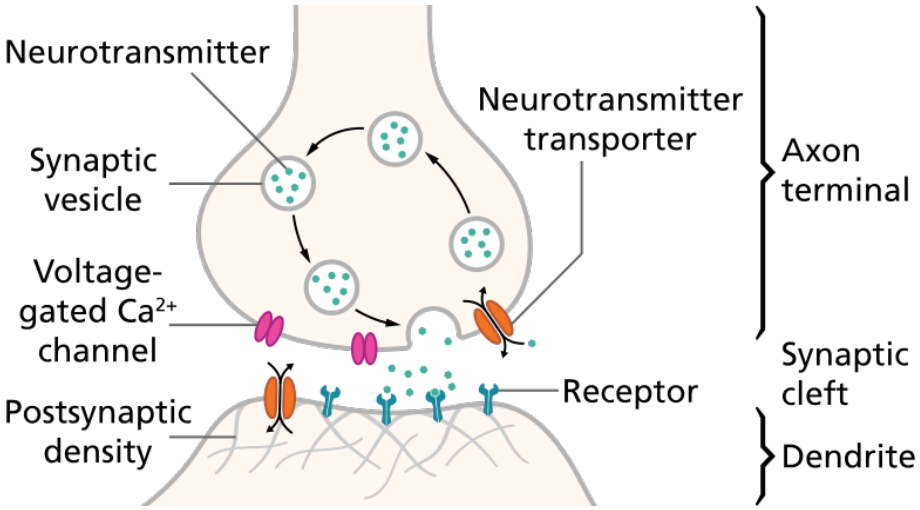


Figure 1.4 – Schema of an electrochemical synapse.

1.2.4. Blood-brain barrier

Blood vessels bring blood to each tissue throughout the body, which is essential to deliver oxygen and nutrients and remove metabolic waste. The blood brain barrier (BBB) is a term used to describe the unique properties of the microvasculature of the CNS[7].

Brain blood vessels are made up of two main cell types: endothelial cells (ECs) that form the walls of the blood vessels, and mural cells on the abluminal surface of the endothelial layer. The properties of the BBB are largely manifested within the ECs, but also by critical interactions with mural cells, immune cells, glial cells, and neural cells. Mural cells include vascular smooth muscle cells that surround the large vessels and pericytes. CNS pericytes have been shown to have unique properties compared to pericytes in other tissues. They contain contractile proteins and are able to contract in order to control the diameter of the capillary[8].

CNS ECs are held together by tight junctions, which creates a barrier to molecules

6. Lodish et al., “Neurotransmitters, Synapses, and Impulse Transmission”.
7. Wolburg and Lippoldt, “Tight junctions of the blood–brain barrier”.
8. Daneman and Prat, “The Blood–Brain Barrier”

and ions. These cellular adhesions are formed on the apical part of the lateral membrane by interactions of transmembrane molecules. CNS ECs express two main categories of transporters: efflux transporters to transport various lipophilic molecules and specific nutrient transporters that facilitate the transport of highly specific nutrients across the BBB into the CNS. The localization in the brain of the latter raises the question about the BBB properties across different regions of the brain. Localized transport of specific nutrients (sugars, amino acids, vitamins, and trace elements) could be important for the development or functions of specific neuronal sub-classes.

1.3. The executive functions

At the most basic level, the function of the nervous system is to send signals from one cell to others, or from one part of the body to others. At a more integrative level, the primary function of the nervous system is to control the body. Nevertheless, better understanding the way neurons encode sensory and high-level information to control behavior, memory and attention is still an area of research. Neurosciences define executive functions, as part of cognitive functions, necessary to control cognition and behavior, to focus and pay attention.

1.3.1. Definition, functions and development

Executive functions are built for reasoning, solving problems, and planning; skills essential in school and in life, as well as cognitive, social, and psychological development.
Executive functions are impaired in many mental disorders, including addictions, attention-deficit/hyperactivity-disorder (ADHD), obsessive-compulsive disorder, and schizophrenia. An efficient executive functioning predicts math and reading competence at school age[9]. There is general agreement that there are three aspects in cognitive control:

- Inhibition and interference control (including selective attention, cognitive and behavioral control). It sets priorities and resistance to impulsive actions or responses.
- Working memory governs the ability to retain and use information over relatively short periods.
- Cognitive flexibility controls the ability of mental set shifting.

These functions are highly interrelated, and the successful application of executive function skills requires them to operate in coordination with each other. Their inte-

9. Diamond, “Executive Functions”.

gration makes it difficult to distinguish them in pathological processes or neuropsychological testing. For example, an alteration of working memory could threaten indirectly cognitive flexibility by reducing the ability of a subject to memorize rules.
The executive functions are among the last brain functions to reach maturity. Attentional control emerges in infancy and develops rapidly in early childhood. Inhibitory control and working memory are among the earliest executive functions to appear, between ages 7 to 12 months. In the preschool years, children improve performances on tasks of inhibition and working memory and maturation continues through adolescence. Cognitive flexibility, goal setting, and information processing usually develop rapidly during ages 7 to 9 years old and mature by 12 years old[10].
During adolescence, systems become more and more integrated and executive functions, such as inhibitory control, are implemented more efficiently and effectively. The major change that occurs is the myelination of neurons in the prefrontal cortex.
In their twenties, adult executive functioning skills are at their maximum. Working memory and spatial span are areas where decline is most readily noted. Cognitive flexibility does not usually start declining until around age 70 years in healthy adults.

In the context of our population, inhibitory control was the executive function of interest, further detailed hereafter.

1.3.2. Inhibitory control

Early in life, inhibitory control skills appears to be quite predictive of outcomes throughout life, including during adulthood. Children who had better inhibitory control at age 3 years were more likely enrolled in school as teenagers and less prone to risky behavior (e.g. smoking or taking drugs). This tendency was still reported in adulthood[11].
The concept of inhibition was introduced at the beginning of the 19th century to explain various neurological phenomena, from spinal reflex to more abstract psychological processes. In neurosciences, the concept of inhibition is commonly used since the fifties but its precise definition is still in debate. In 2013, Bari and Robbins proposed two kinds of inhibition: cognitive and behavioral[12].
According to this distinction, the first one encompasses inhibiting prepotent mental representations, resisting to unwanted thoughts, memories, or pre-existent information acquired earlier. It usually serves working memory and is more or less conscious[13]. On the other hand, behavioral inhibition is voluntary and more linked to

10. Diamond et al., “Preschool Program Improves Cognitive Control”; Luna et al., “What has fMRI told us about the development of cognitive control through adolescence?”
11. Moffitt et al., “A gradient of childhood self-control predicts health, wealth, and public safety”.
12. Bari and Robbins, “Inhibition and impulsivity”.
13. Hasher and Zacks, “Working Memory, Comprehension, and Aging”.

attention (and also called executive attention or attentional inhibition). They further subdivided behavioral inhibition in three components: response inhibition, deferred gratification and reversal learning, which enable to ignore particular stimuli and refrain, suppress or delay a prepotent response.

Response inhibition is the easiest aspect of inhibitory control to evaluate and can be studied directly through several neuropsychological tasks, some of which are listed below:

- The Simon task consists of two rules: for stimulus A press on the left; for stimulus B press on the right. It investigates response inhibition by initiating a prepotent response.

- The Spatial Stroop task is similar to a Simon task but minimizes memory demand because the stimulus shows where to respond with an arrow pointing in the direction to press. Sometimes the arrow appears on the side it is pointing toward (congruent, compatible trials), but sometimes the arrow appears on the other side (incongruent, incompatible trials).

- The Conners' Continuous Performance Test (CPT) where the participant is asked to press when a letter other than X appears.

- The Inhibition Scale of the developmental NEuroPSYchological assessment (NEPSY) where a series of black and white shapes or arrows is presented. The rule is to name either the shape or direction or an alternate response, depending on the color of the shape or arrow.

- The Color Word Interference Test of the D-KEFS, based on the Stroop procedure.

- The Go/No-Go tasks, similar to the CPT: the subject is asked to press a button when a stimulus appears, but to not press when another certain stimulus appears.

- The Stop-Signal task: the go signal is presented on all trials; on a minority of trials after the go signal and just as the subject is about to respond, a stop signal appears (usually a sound), indicating that one should not press the button on that trial.

The CPT, Go/No-Go and Stop-Signal tasks are different from other measures in that participants do not inhibit one response to make another. They only inhibit a response, limiting cognitive demand other than inhibition.

1.4. Visualizing brain with cerebral imaging

1.4.1. Structural and functional imaging

Neuropsychological testing is efficient to investigate specific behavioral and cognitive functions but they give little information about neural basis. Brain imaging techniques allow investigating the structure and function of the human brain, to provide complementary information to neuropsychological evaluations. Neuroimaging is divided into two main categories: structural imaging provides information about the volumes, shapes or connections between the structure of the brain, while functional imaging allows visualizing neural, neurovascular or metabolic processes. Techniques using ionizing radiations or incompatible for research purposes are not presented here.

Structural imaging

Structural imaging provides information about the volumes, shapes or connections between structures, relying on contrast (i.e. differences in gray level) to infer information about the type of tissue (WM, GM, cerebrospinal fluid (CSF), subcutaneous fat, skull and potential lesions or abnormalities). These images of different contrasts can be assessed radiologically for diagnostic purposes and numerically, to compute volume or shape metrics. These computations can be performed at a regional or voxel level using Voxel-Based Morphometry (VBM). Magnetic Resonance Imaging (MRI) scans are registered (i.e. normalized) to a common space (i.e. template) and combined to an average brain. Specific anatomical differences of an individual can be compared to others. This technique is very sensitive to brain structures, tissues classification and folding patterns.
Structural imaging also encompasses diffusion tensor imaging (DTI), which is sensitive to the motion of water molecules and potential diffusion restriction along the white matter fiber bundles. This technique allows mapping the white matter tracts that connect regions of the brain with one another.

Brain function imaging

Brain function can be evaluated using electroencephalography, or EEG, the measurement of the electrical activity of the brain. This technique offers a high temporal resolution, capable of detecting changes in electrical activity in the brain on a millisecond-level. Similarly, magnetoencephalography, or MEG, uses magnetic fields produced by electrical activity in the brain. MEG measures directly neural electrical activity with a very high temporal resolution but relatively low spatial resolution.

Brain functional MRI (fMRI) and functional near infra-red spectroscopy (fNIRS) use the changes in neurovascular coupling in blood oxygenation associated to neural activity to map brain regions involved in a specific cognitive task, or at rest. This

technique is non-invasive, has a spatial resolution of a few millimeters but poor temporal resolution (1 to 3 seconds).
fNIRS is an optical technique which, like fMRI, measures blood oxygenation changes in the brain. Near infrared lights are sent through the skull, reemerging lights are then detected and their attenuation provide information about oxygenation levels, from which neural activity is inferred. Compared to fMRI, this technique is more portable and less expensive, but is limited to the cortical surface.
Event-related optical signal, or EROS, uses infrared light via optical fibers to assess alterations in optical properties in active regions of the brain. It assesses scattering of neurons, generating an accurate portrayal of neuronal activity. This recent technique is relatively inexpensive and directly measure neural activity. Like fNIRS, it is unable to detect activity outside the cortex surface.

fMRI was the technique used in the present study to visualized brain function because it allowed to image whole-brain function non-invasively with a fine spatial resolution. Its principles will be further detailed.

1.4.2. Physical principles of MRI

Physical principles of MRI are further detailed in Appendix on page 193.
An atomic nucleus with an odd number of protons and/or neutrons (e.g. hydrogen, one unique proton and no neutron) have a "spin" property. The nucleus spins around its own axis, generating a local magnetic field (cf. Figure 1.5).

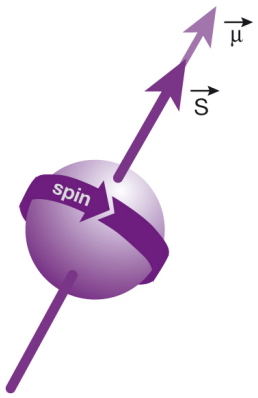


Figure 1.5 – Magnetic moment[21].

In presence of an external magnetic field, nuclear spins are distributed into two energy states. There is a small excess of spins in the low-energy state (vs. high) sufficient to produce a nuclear magnetic resonance (NMR) magnetization at tissue level.
Within the static magnetic field, nuclei can be excited by the application of a second rotating radiofrequency magnetic field, perpendicular to the first one, during short

pulses of a few microseconds. Nuclei absorb the energy and transit from lower to higher energy states. In the absence of continued pulsation, the system returns to equilibrium in a process called relaxation. There are two components of relaxation, longitudinal and transverse, described by the time constants: T1 and T2, respectively. These constants are specific to a given tissue at a given field strength.
Image contrast can be manipulated by varying the gradient and radiofrequency pulse shapes and the time between pulses (i.e. pulse sequence). Conventional T1 and T2 weighted images derive from different pulse sequences and timing. Besides, minor differences in chemical environment disturb magnetic susceptibility and transverse relaxation, resulting in T2* relaxation.

1.4.3. Physiological principles of BOLD functional MRI

While structural MRI can provide a single 3D high-resolution representation of the brain, functional imaging requires acquiring a series of 3D volumes with a high temporal resolution (a few seconds) at the expense of a slightly lower spatial resolution. Blood oxygenation level dependent (BOLD) fMRI uses a series of T2* weighted echo planar images which can be acquired with high temporal resolution and are sensitive to oxygen concentration.
Indeed oxygenated hemoglobin (oHb) is diamagnetic and does not disturb the signal, whereas deoxygenated hemoglobin (dHb) is paramagnetic and decreases T2* signal. When a brain region is activated, an increased blood flow brings more glucose and more oxygen, i.e. more oHb molecules in red blood cells, to the brain. This higher rate of blood flow and expansion of blood vessels is the haemodynamic response (HR), allows rapid delivery of blood to active neuronal tissues. Oxygen need is overcompensated, resulting in an increase of the ratio oHb/dHb and therefore an increased magnetic signal in that brain region. The signal variation is small (around 5% of total signal), transitory (2-10 seconds) and localized (within 2 or 3 mm of where the neural activity is).
Even at rest, the whole brain is active. To be specific of a task, findings are usually reported as degree of activation or the results of subtraction contrasts, typically (active condition) - (control condition).
There are two major types of experimental designs in task-based fMRI: blocked and event-related designs. In a block design, a condition is presented continuously for an extended time interval (block) to maintain cognitive engagement, and different task conditions are alternating in time. Block design are robust, present a relatively large BOLD signal and a strong statistical power[14]. During event-related designs, events are presented in a discrete and short-duration fashion, with randomized interstimulus intervals to allow the HR response to return towards baseline. They allow anal-

14. Tie et al., “Comparison of blocked and event-related fMRI designs for pre-surgical language mapping”.

ysis of individual responses to trials and can be randomized to reduce subject's habituation. The inter-stimulus-interval is a key parameter in event-related designs. A rapid event-related design, shorter than the duration of the haemodynamic response function (HRF, 10–12 s), is similar to those used in the classical neuropsychological experiments and increases the statistical power by allowing for more stimuli per time unit.

1.4.4. BOLD responses associated with inhibitory control

There is partial agreement across several neuroimaging studies about neural basis of executive functions. The prefrontal cortex is a key structure involved in verbal and design fluency, maintaining and shifting attention, planning, inhibitory control, working memory, organizational skills, reasoning and problem solving. However, it is not sufficient for response inhibition and other cortical and subcortical regions are needed[15]. Brain imaging studies have found overlapping active regions for both cognitive and response inhibition paradigms. It has been shown that motor response inhibition can be used as a *proxy* to investigate neural underpinnings of inhibition[16].

Historical development of research on inhibition

As synthesized by Bari and Robbins in 2013, at first, scientific studies focused on searching for a locus of inhibition in the nervous system. Some thought to discover a central inhibitory locus in the brain stem, while others concluded that there are no specific inhibitory centers, but higher centers repress lower ones. Others proposed an "intracellular" theory of inhibition where stimuli that reach the central part of a nerve cell have inhibitory effects, whereas those reaching the periphery are predominantly excitatory; and thus no need of special inhibitory centers[15].
In the sixties, animal experiments reported that frontal lesions produce abnormal difficulty in suppressing whatever response normally prevailing in a given situation, disinhibition of inhibitory reflexes, distractibility and over-reactivity. However, the pattern of deficits was not precisely defined. In addition, bilateral ablation of the striatum was found to have effects similar to those of frontal lesions, which suggests that the striatum forms a link in the inhibitory path from the cortex to the motor regions[15].
With the development of *ad hoc* tasks that explicitly measure the ability to suppress prepotent responses rather than automatic reflexes, scientists began to explore more defined parts of inhibition. Since, evidence of the inhibitory function of frontal areas has been reported in human subjects by using a variety of techniques such as MEG, EEG, event-related potentials and Positron Emission Tomography (PET).

15. Bari and Robbins, "Inhibition and impulsivity"
16. Mostofsky et al., "fMRI evidence that the neural basis of response inhibition is task-dependent"; Suskauer et al., "Functional Magnetic Resonance Imaging Evidence for Abnormalities in Response Selection in Attention Deficit Hyperactivity Disorder".

Brain regions involved in the inhibition network

Depending on the inhibition task and the requirement of ancillary resources (e.g. working memory, selective attention), different cortical and subcortical brain regions are involved (cf. Figure 1.6).
Frontal and inferior parietal lobes mediate performance on response inhibition, regardless of the task. However, it seems that in Go/No-Go tasks the selective inhibition is associated with a bilateral, mostly in the left middle-infero-mesio-frontal and parietal network. Meanwhile withholding a planned motor response in Stop-Signal tasks elicits a predominantly right hemispheric network[17].
Liddle *et al.* used event-related fMRI to measure the HR during a Go/No-Go task in 16 healthy subjects with equally probable go and no-go events. Anterior cingulate was active during both the go and no-go trials, dorsolateral and ventrolateral prefrontal cortex was more active during the no-go events, while primary motor cortex, supplementary motor area (SMA), premotor cortex and cerebellum were more active during go events. These findings suggest that the anterior cingulate cortex is primarily engaged in making and monitoring of decisions, while dorsolateral and ventrolateral prefrontal sites play a specific role in response inhibition[18].
Indeed, activation of the right inferior frontal cortex and the right insula has been consistently observed during response inhibition tasks, although activation is sometimes observed bilaterally. However, the insular cortex may be involved in conflict resolution and maintaining high levels of motivation rather than stopping[19]. There is also a controversy on the role of inferior frontal cortex since some studies failed to link this locus to response inhibition. Right inferior frontal cortex may be involved in response inhibition on its ventral part only[20]. Further studies confirmed that the dorsolateral part of the prefrontal cortex is probably more involved in maintaining task rules as its activation has been related to the increased working memory load[21].

Other regions are also needed to perform ancillary processes of response inhibition. Excision in the dorsomedial cortex was reported to lead to an increase of commission rate during a Go/No-Go task, suggesting that the anterior cingulate, SMA and pre-SMA regions may be involved in inhibitory impairments[22].
The SMA is probably more involved in response initiation and selection than stop-

17. Rubia, Russell, et al., “Mapping Motor Inhibition”.
18. Liddle et al., “Event-related fMRI study of response inhibition”.
19. Droutman et al., “Roles of the Different Sub-Regions of the Insular Cortex in Various Phases of the Decision-Making Process”.
20. Bari and Robbins, “Inhibition and impulsivity”; Dosenbach et al., “Distinct brain networks for adaptive and stable task control in humans”.
21. Mostofsky et al., “fMRI evidence that the neural basis of response inhibition is task-dependent”; Simmonds et al., “Meta-analysis of Go/No-go tasks demonstrating that fMRI activation associated with response inhibition is task-dependent”.
22. Chambers et al., “Insights into the neural basis of response inhibition from cognitive and clinical neuroscience”

ping process[23]. Anterior cingulate cortex has a possible role in response inhibition with error detection, conflict monitoring and working memory. Therefore, the human anterior cingulate cortex has been proposed to subserve motor control by facilitating appropriate responses and allocation of attentional resources[24].

Current models of inhibitory control acknowledge the critical contribution of prefrontal and premotor areas to response inhibition and consider the orienting reaction to be modulated by a brain stem-subcortical circuit. In general, executive control is thought to operate in a hierarchical manner with the prefrontal cortex having a leading role over lower-level structures[22].
In summary, given the number of different processes necessary for successful response inhibition, both in real life and in the laboratory (e.g., stimulus/conflict detection, sustained attention, attentional shifting, error monitoring), it seems likely that a complex circuit is involved. It is probably the interaction between inferior frontal cortex and pre-SMA that allows the successful inhibition of a prepotent motor response[25].

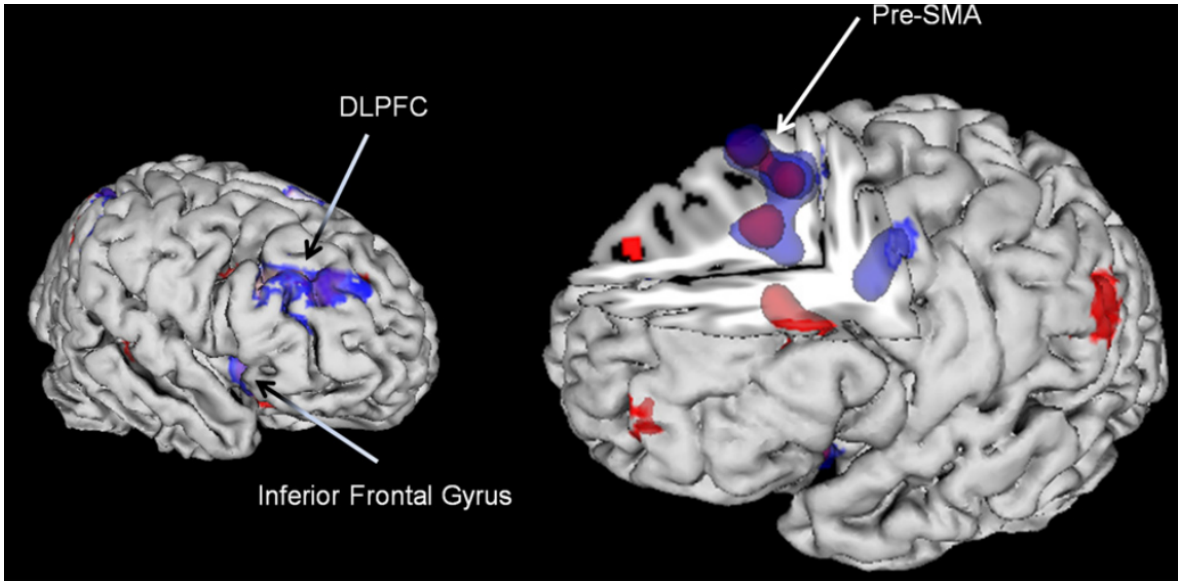


Figure 1.6 – Brain regions involved in inhibition[23].

Factors associated with activations of the inhibition network

The fMRI literature characterizing age-related differences in cognitive control has grown over the last decades. This work has generated diverse findings that still share some important consistencies. Results indicate both childhood increase in prefrontal, striatal and parieto-temporal participation and a decrease in posterior and

23. Criaud and Boulinguez, “Have we been asking the right questions when assessing response inhibition in go/no-go tasks with fMRI?”
24. Simon et al., “Anterior cingulate cortex and intuitive bias detection during number conservation”.
25. Aron, “The Neural Basis of Inhibition in Cognitive Control”.

limbic regions. This increased activation in children and adolescents suggests that performing executive tasks may require increased effortful attention of the youths, therefore necessitating more resources. These results could also suggest progressive integration of both task-positive fronto-cortical and fronto-subcortical activation[26].

We might expect sex-specific activation during response inhibition because differences between males and females are observed in pathological disorders (e.g. ADHD). Nevertheless, findings regarding sex differences of the neural basis of inhibition are not conclusive[22].

Response-inhibition deficits have been linked to several psychopathological and neurological disorders by neuroimaging studies. Some disorders are associated with general cognitive impairments (such as autism or schizophrenia), as well as inhibitory deficits. Other disorders, such as ADHD and compulsive disorders, are described specifically as inhibitory disorders. Children with ADHD do not activate fronto-striatal regions in the same manner as normally developing children, but rather rely on a more diffuse network of regions, including more posterior and dorsolateral prefrontal regions[27].

Functional MRI studies seem to be an ideal tool to study brain development and possible alterations. These alterations may occur during the large period of neurodevelopment.

1.5. Embryogenesis of the brain and neurodevelopment

The brain is one of the earliest systems to begin and the last to be completed after birth. At embryonic stages, landmarks of neural development include the neurogenesis from stem cell precursors, the migration of immature neurons, the growth of axons and the generation of synapses. After birth, synaptic pruning occurs in adolescence, and then lifelong changes in synapses control brain plasticity.

1.5.1. Prenatal development

Neurulation

First, cells migrate to the interior of the embryo, forming three layers: the endoderm (the deepest layer), mesoderm and ectoderm (the surface layer) during gastrulation. During the third week of gestation, the notochord, formed from the mesoderm, sends chemical signals to the overlying ectoderm, inducing it to become neuroectoderm.

26. Chambers et al., “Insights into the neural basis of response inhibition from cognitive and clinical neuroscience”; Rubia, “Functional brain imaging across development”.

27. Durston et al., “Differential patterns of striatal activation in young children with and without ADHD”; Konrad et al., “Dysfunctional Attentional Networks in Children with Attention Deficit/Hyperactivity Disorder”; Suskauer et al., “Functional Magnetic Resonance Imaging Evidence for Abnormalities in Response Selection in Attention Deficit Hyperactivity Disorder”; Yang et al., “Abnormal spontaneous brain activity in medication-naïve ADHD children”.

This results in a neural plate of neuronal stem cells that runs along the back of the embryo. The neural plate folds outwards to form the neural groove (cf. Figure 1.7). Beginning in the future neck region, the neural folds of this groove close to create the neural tube. By the end of the fourth week of gestation, the open ends of the neural tube closes off.

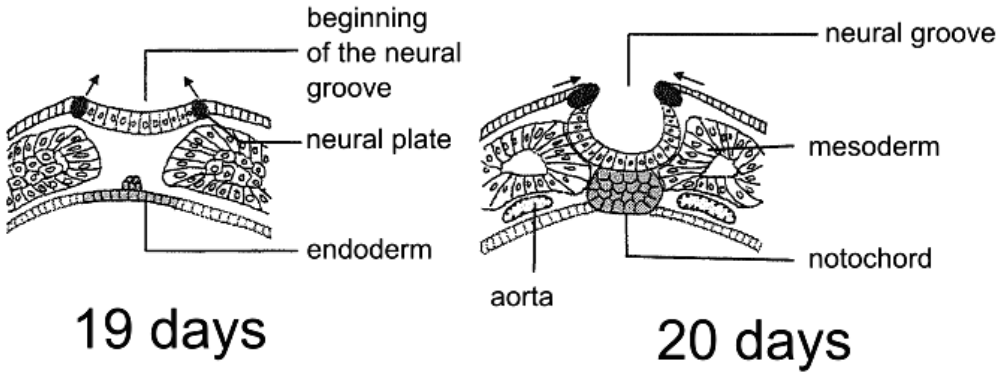


Figure 1.7 – Cross-sectional view of the formation of the neural groove [Adapted from Pradal, *Embryologie humaine élémentaire*].

Proliferation

Neuronal proliferation in the CNS occurs within the ventricular lining of the original neural tube or its later derivatives in distinct spatio-temporal patterns (from the 2nd months of gestation to age 2 years). Because of the absence of markers for the different lineages of neuronal stem cells, little is known about the factors regulating the pattern of cell divisions.

Neural migration

Neuronal migration is the method by which neurons travel from their birthplace to their final position in the brain (from gestational months 2 to 6). Their most common means of migration are radial and tangential migration. Each wave of migrating cells travel past their predecessors forming layers in an inside-out manner, meaning that the youngest neurons are the closest to the surface. It is estimated that glia guided migration represents 80-90% of migrating neurons.

Differentiation

Cells acquire many of the differentiated features characteristic of neurons or glia during the migration phase, but their major phase of differentiation usually occurs only after they have reached their final destinations. First, the cells acquire a distinctive morphology, usually characterized by the development of several dendrites and

a single axon. Second, the cells acquire a number of distinctive membrane properties. These properties generally appear over a period. Then, the neuron adopts a particular mode of synaptic transmission. In most neurons, the cells generate all the necessary cellular machinery for their transport to the axon terminals and for their exocytotic release. Simultaneously, the cells express a variety of receptor molecules that become inserted into the appropriate postsynaptic sites on their own surfaces.

Axonal growth

Axons are extensions of the cell that grow by the addition of new materials at their expanded ends, referred to as growth cones. Most axons do not grow in isolation but in association with other axons from the same neuronal population. A different set of factors seems to be involved in the identification of the desired target, including chemoaffinity. According to this hypothesis, each small group of neighboring neurons requires a distinctive cytochemical label expressed on the surfaces of the growing axons and the presence of matching or complementary labels on the target cells enabling the axons to recognize and to form synapses with their appropriate partners.

Synapse formation

The final event in the establishment of the initial pattern of connections is the formation of synaptic contacts between the related populations of cells (from 2^{nd} months of gestation to age 10 years). The assembly of the entire complement of presynaptic and postsynaptic components involve a complex set of inductive interactions between the axon terminal and its target cell.

Apoptosis

Neurons are initially overproduced. At some later period, between 15 and 85% of the initial population degenerate. Because growing axons have to make their way through a veritable jungle of other neuronal and glial cells, some of them process through an incorrect pathway or grow to an inappropriate target. The finding that the number of finally survivor neurons is closely related to the size of the target field has led to the hypothesis that the neuron axons compete with each other.

Formation of the blood-brain barrier

At embryonic stage, neural stem cells appear to be the key cell type involved in the early differentiation of the ECs into BBB ECs, and then pericytes and astrocytes provide further cues modulating the properties of these CNS ECs. The BBB is not a single entity, but a series of different properties possessed by the CNS ECs. Therefore, it seems likely that the fetal BBB is impermeable to macromolecules even at

early stages. In contrast, small molecules access fetal brains more easily than they do adult brains[28].

1.5.2. Postnatal development

The production and migration of neurons are largely prenatal events, proliferation and migration of glial progenitors continues during the first years after birth, and the differentiation and maturation of these cells continue throughout childhood.
Although the development of neural networks requires the formation of precise connections between developing neurons and their targets, it is well documented that initial patterns of connectivity in the developing brain are exuberant in terms of both the numbers of formed connections and their topography and will eventually disappear.

1.5.3. Vulnerable periods

During those critical periods of neurodevelopment, the brain is thought to be particularly vulnerable to its environment and disruption of development processes can lead to neurodevelopmental diseases or disorders[29]. In the literature, two vulnerable periods are particularly studied. The first includes its early development, during neurogenesis and alteration may result in severe brain anomalies (e.g. anencephaly, spina bifida). The second period is during the brain growth spurt occurring during childhood, with maturation of axonal and dendritic outgrowth, and synaptogenesis and myelination (cf. Figure 1.8).

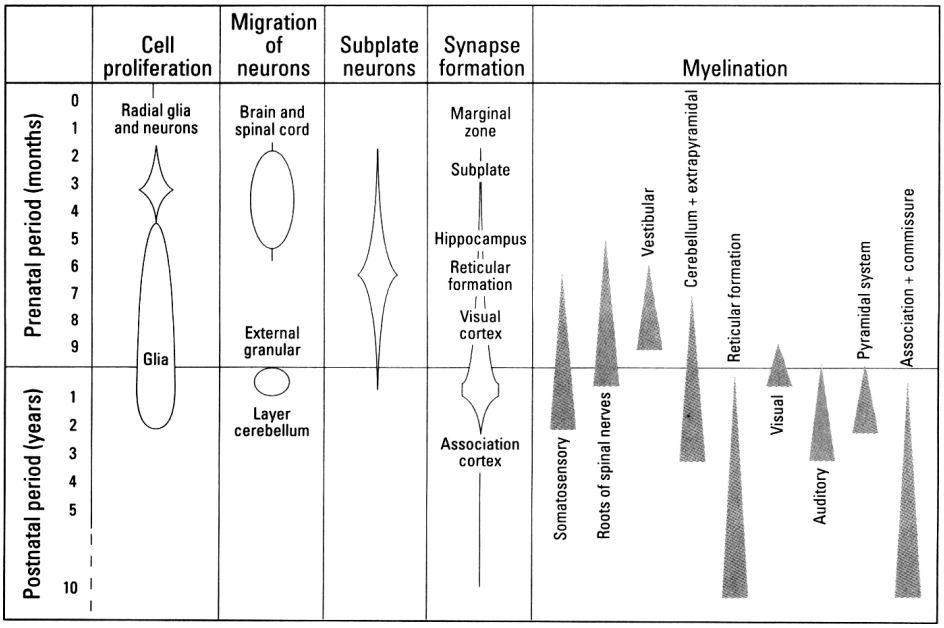


Figure 1.8 – Windows of development of the human brain[29].

28. Daneman and Prat, “The Blood–Brain Barrier”.
29. Rice and Barone, “Critical periods of vulnerability for the developing nervous system”

2.1. Generalities about behavioral and cognitive disorders

Neurodevelopmental disorders are abnormalities in the brain that affect behavior, memory or ability to learn of a child and last for a person's entire lifetime. The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) currently considers neurodevelopmental the following disorders: intellectual disability, global developmental delay, specific learning disorders, autism spectrum disorders, ADHD, motor disorders and communication disorders (including language, speech sound, childhood-onset and social communication)[1].
Symptoms, acquired brain injuries or neurological diseases are usually defined and diagnosed based on their symptomatology rather than etiology. For example, Parkinson's disease, a neurodegenerative condition, and ADHD, a neurodevelopmental disorder, have different causes but are both characterized by executive dysfunction. Among neurodevelopmental disorders, ADHD and autism symptoms suggest executive function alterations.

In France in 2011, Lencendreux *et al.* estimated that 3.5 to 5.6% of children were diagnosed with ADHD. In the USA, according to parent reports in 2016, 9.4% of children (6.1 million) between 2 and 17 years of age had been diagnosed with ADHD (criteria of diagnosis are detailed in Appendix on page 195)[2].
People with ADHD often have problems with organization, discipline, and setting priorities, and these difficulties often persist from childhood through adulthood.
Although ADHD has typically been conceptualized in a categorical diagnosis paradigm, it could be considered within a more dimensional behavioral model that links executive functions to observed deficits. The normal development of inhibition is essential for the performance of other executive functions, such as working memory and emotional self-regulation. Thus, developmental deficits in inhibition may result in dysfunctional executive systems in later life.
Previous studies have suggested the possibility that the severity of executive dysfunction in individuals with ADHD declines with age. Nevertheless, a decrease in executive dysfunction in adults with ADHD as compared to children with ADHD is thought to reflect compensatory strategies employed by adults (e.g. using schedules to organize tasks) rather than neurological differences[3].

The incidence of autism spectrum disorders was estimated to 1/100 births in 2012 in France and 1/59 children in 2014 in the USA. Autism is diagnosed based on the presence of markedly abnormal or impaired development in social interaction and

1. *Diagnostic and statistical manual of mental disorders (5th ed.)*
2. Lecendreux et al., "Prevalence of Attention Deficit Hyperactivity Disorder and Associated Features Among Children in France".
3. Kysow et al., "The use of compensatory strategies in adults with ADHD symptoms".

communication and few activities and interests[4].
Due to the variability of symptoms and functions impaired, the disorder is typically conceptualized as a spectrum of severity. Individuals with autism commonly show impairment in three main areas of executive functioning[1]:

- Fluency, autistic children show impairments in generating novel ideas and produce less complex responses than typically developed children.
- Planning, persons with autism demonstrate impairment on tasks requiring planning abilities and complex, dynamic processes.
- Poor mental flexibility, individuals with autism are characterized by perseverative and stereotyped behavior, and deficits in both the regulation and modulation of motor acts.

Although there has been some debate, response inhibition does not seem altered in people with autism. Individuals with autism have demonstrated similar performance on various tests of inhibition.

Specific learning disorder is a neurodevelopmental disorder that begins at school-age. Learning disabilities refers to ongoing problems in reading, writing and/or math. In 2011-2012 in the US, 8.0 percent of children of ages 3–17 presented learning disabilities. Reading disorder, also referred to as dyslexia is the most common disorder and around 1/3 of people with ADHD were also diagnosed with reading disorders[5]. Specific skills that may be impacted include the ability to put thoughts into written words, spelling, reading comprehension, math calculation and math problem solving. Difficulties with these skills may cause problems in learning subjects such as history, math, science and social studies and may affect everyday activities[6].

2.2. The brain and environment

Recent epidemiological data indicate that prevalence rates of neurodevelopmental disorders have increased in the last two decades[7]. Although such increase may reflect increased awareness of these disorders and broader diagnostic criteria, there is general concern on the possible implication of environmental factors in the etiology of neurodevelopmental disorders[8]. According to the National Academy of Sciences of the United States, 3% of neurodevelopmental troubles may result from exposure to neurotoxicant substances and 25% may be the consequence of an interaction

4. Baio, “Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014”.
5. Germanò et al., “Comorbidity of ADHD and dyslexia”.
6. Boat and Wu, *Prevalence of Learning Disabilities*.
7. Boyle et al., “Trends in the Prevalence of Developmental Disabilities in US Children, 1997-2008”.
8. Dulcan, “Practice Parameters for the Assessment and Treatment of Children, Adolescents, and Adults With Attention-Deficit/Hyperactivity Disorder”.

between exposure and individual genetic susceptibilities[9]. Moreover, subclinical effects which are not clinical and diagnosed troubles have also a significant impact at population level in terms of public health (e.g. lower Intelligence Quotient (IQ))[10].

Since the development of the nervous system including the brain is orchestrated by genetically encoded processes, the literature suggests that any perturbation of this program early in life can result in neurodevelopmental disorders and, depending on specific timing, might lead to distinct pathologies later in life[11] .
Identification of causative factors for neurodevelopmental alterations is a difficult task, resulting from multiple etiologies and/or mechanisms. In human studies, exposure to mixtures of chemicals or to different risk factors makes it difficult to isolate the contribution of the different chemical (or not) factors to the etiology of the disease[12].
In this way, a Menorca's birth study about social and environmental exposures in early childhood reported that both social, nutritional characteristics (e.g. parental social class, educational level, maternal nutritional supplementation with folic acid and vitamins and breastfeeding) and environmental exposures to organochlorine compounds might influence inattentive and hyperactive/impulsive symptomatology during pre-adolescence[13].

2.2.1. Pregnancy and childhood medical conditions

Prematurity and IUGR

Preterm infants have been reported to be at particular risk of long-term cognitive difficulties, psychiatric disorders (e.g. anxiety) and neurodevelopmental disorders (e.g. attention deficit hyperactivity disorders). In particular, several studies suggested intrauterine growth restriction (IUGR) increased the risk of such cognitive disabilities[14].

Mechanical trauma

Brain trauma lead around 7 people (adults and children) per 1,000 treated in emergency departments each year. In infants and youth, it is a common postnatal cause

9. National Research Council et al., *Scientific Frontiers in Developmental Toxicology and Risk Assessment*
10. Rice and Barone, "Critical periods of vulnerability for the developing nervous system"
11. Grandjean and Landrigan, "Developmental neurotoxicity of industrial chemicals"
12. Eskenazi, Rosas, et al., "Pesticide Toxicity and the Developing Brain"; Grandjean and Landrigan, "Developmental neurotoxicity of industrial chemicals"; Julvez and Grandjean, "Neurodevelopmental toxicity risks due to occupational exposure to industrial chemicals during pregnancy".
13. Forns et al., "Longitudinal association between early life socio-environmental factors and attention function at the age 11 years".
14. Johnson, "Cognitive and behavioural outcomes following very preterm birth"; Murray et al., "Differential effect of intrauterine growth restriction on childhood neurodevelopment"; Réveillon et al., "Functional neuroimaging study of performances on a Go/No-go task in 6- to 7-year-old preterm children".

of neurodevelopmental disorders, occurring after congenital injuries (e.g. asphyxia, hypoxia), during birth delivery or as results of falls during childhood[15].

Metabolic or hormonal disorders

Maternal and child metabolic disorders can cause neurodevelopmental disorders. Excessive maternal phenylalanine can be absorbed by the fetus and produce mental retardation and other neurodevelopmental disorders.
In a child, uncontrolled type 1 diabetes can produce neurodevelopmental damage by the effects of excess or insufficient glucose. Type 2 diabetes during childhood may be associated with early symptoms on cognitive function. A non-diabetic fetus can also be subjected to glucose effects if its mother has undetected gestational diabetes.
Maternal thyroid hormone insufficiency, even at mild levels including subclinical hypothyroidism and isolated hypothyroxinemia have been associated with intellectual disabilities and more inconsistently with ADHD and autism spectrum disorders[16].

Biological factors

Infectious diseases or immune reactions during pregnancy, both maternal and fetal, may produce neurodevelopmental disorders.
A number of infectious diseases can also be transmitted congenitally, in-utero or at delivery, as for example the viruses HSV, CMV, rubella, Zika virus, or bacteria like Treponema pallidum, protozoa like Plasmodium or Toxoplasma and lead to neurodevelopmental alterations.
During childhood, infections of the head and brain, such as brain abscesses, meningitis or encephalitis have a high risk of causing neurodevelopmental disorders.
Then, a typical immune reaction in infants and children is the pediatric autoimmune neuropsychiatric disorders associated with Streptococcal infection (PANDAS) characterized by obsessive-compulsive disorders[17].

2.2.2. Social context

Deprivation

Deprivation from social and emotional care causes severe delays in brain and cognitive development.
Stressful experiences during pregnancy, in particular early pregnancy, can contribute to poor pregnancy outcome. Maternal stress can be divided into three independent risk factors: psycho-social stress, strenuous physical activity, and food

15. Thurman, “The Epidemiology of Traumatic Brain Injury in Children and Youths”.
16. Thompson et al., “Maternal thyroid hormone insufficiency during pregnancy and risk of neurodevelopmental disorders in offspring”.
17. Swedo et al., “Clinical Presentation of Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections in Research and Community Settings”.

deprivation. A physiological role is probably played by the production of the neuropeptide corticotrophin-releasing hormone (CRH) and elevation of cortisol levels under the influence of mental stress. Stress may cause preterm delivery and growth retardation and both are known to have negative influences on neurodevelopment[18]. Studies with children growing up in Romanian orphanages during Nicolae Ceauşescu's regime reveal profound time-dependent effects of social and language deprivations on the developing brain. Indeed, stressful or deprived environments during childhood seem associated with impaired brain structure and function[19].

Nutrition

The most common nutritional cause of neural tube defects is maternal folic acid deficiency, a B vitamin usually found in fruits, vegetables, whole grains, and milk products. Deficiency in iodine, a key nutrient for thyroid metabolism, produces a spectrum of neurodevelopmental disorders ranging from mild emotional disturbance to severe mental retardation. Excesses in both maternal and infant diets may cause disorders as well. Iron supplementation in baby formula has also been linked to lowered IQ[20].
The Dutch Hunger Winter studies are a good example for the consequences and the outcome later in life of food deprivation in early pregnancy. Perinatal mortality and congenital malformation rate like spina bifida and anencephaly are increased in people conceived during the height of the famine. There is a peak in schizophrenia in the babies born in November 1945, so conceived in the spring, when problems were the most severe. It has to be pointed out that mothers were not only deprived of food, but were also living under stressful war conditions[21].
Even if the effects of nutrition during adulthood is not as much studied than infancy period, it seems likely that postnatal diet may affect the development of the brain and its function[22].

2.2.3. Chemicals

In 2006, a review identified around 200 chemicals known to be toxic in adults, but only about 20 have been investigated for neurodevelopmental effects[23]. Roughly, neurodevelopmental effects of chemical exposure can result from parental behavior during pregnancy (e.g. drugs, tobacco or alcohol consumption) or presence in environment in-utero or during childhood.

18. Réveillon et al., "Functional neuroimaging study of performances on a Go/No-go task in 6- to 7-year-old preterm children".
19. Brietzke et al., "Impact of childhood stress on psychopathology"; Sheridan and McLaughlin, "Dimensions of early experience and neural development".
20. Sinn, "Nutritional and dietary influences on attention deficit hyperactivity disorder".
21. Susser et al., "Neurodevelopmental Disorders after Prenatal Famine".
22. Goyal et al., "Brain Nutrition".
23. Grandjean and Landrigan, "Developmental neurotoxicity of industrial chemicals"

Drugs

A Dutch follow-up study about children in which 80% of mothers used heroin, methadone or both and 20% used other psychoactive drugs reported a high percentage of premature birth between 32-36 weeks and infants small for gestational age at birth. Behavioral problems were also increased and social capabilities were lower than normal[24]. Yolton *et al.* reviewed literature about prenatal exposure to cocaine or heroin and reported links between exposure, behavioral troubles and cognitive alterations. They pointed out the lack of studies and methodological issues to precisely measure drug use[25]. Cannabis use during pregnancy can cause adverse effects on neurodevelopment[26]. Moreover, there is some evidence that use in adolescence can facilitate the manifestation of schizophrenia[27].

Smoking

Smoking leads both to fetal nicotine and carbon monoxide exposure. Both maternal and passive smoking are associated with behavior disorders and poorer academic achievements, and more inconsistently with psychomotor development[28].

Alcohol

Alcohol consumption during pregnancy has been previously associated with reduced birth weight, which could mediate an alteration on neurodevelopment. Heavy levels of consumption lead to fetal alcohol syndrome, characterized by abnormal appearance, growth delay, learning and behavioral disabilities[29]. Studies about low or moderate levels of exposure to alcohol also suggest that consumption of alcohol during pregnancy may adversely affect cognitive skills, mental health, memory and verbal or visual performance[28].

Heavy metals

Environmental exposure to heavy metals, such as lead, methylmercury and arsenic are associated with neurodevelopmental alterations[30].
Lead (Pb) mimics Calcium action in cells. Prenatal exposure to Pb has been associated with cognitive impairments in children but the most significant effects (e.g.

24. Baar et al., “Development after prenatal exposure to cocaine, heroin and methadone”.
25. Yolton et al., “Exposure to neurotoxicants and the development of attention deficit hyperactivity disorder and its related behaviors in childhood”.
26. Bara et al., “Sex-dependent effects of in utero cannabinoid exposure on cortical function”.
27. Manrique-Garcia et al., “Cannabis, schizophrenia and other non-affective psychoses”; Smit et al., “Cannabis use and the risk of later schizophrenia”.
28. Polańska et al., “Smoking and alcohol drinking during pregnancy as the risk factors for poor child neurodevelopment – A review of epidemiological studies”
29. Institut national de la santé et de la recherche (INSERM), *Alcool*.
30. Grandjean and Landrigan, “Developmental neurotoxicity of industrial chemicals”; Vrijheid et al., “Environmental pollutants and child health—A review of recent concerns”.

lower IQ and ADHD symptoms, such as distractibility, difficulties to inhibit and perseverative behavior) have been observed after childhood exposure, even at low concentration.
Similarly, perinatal exposure at low levels of methylmercury (Hg) has been associated with adverse neurodevelopmental outcomes, such as autistic spectrum disorders or impaired cognition[31]. Since exposure to Hg occurs mainly through fish consumption, the protective of fatty acids on brain function may counterbalance detrimental neurodevelopmental effects of Hg.

Concerning Manganese (Mn) and Cadmium (Cd) perinatal exposure, a systematic review suggested adverse effects of Cd on cognitive abilities but little evidence of behavioral effects. However, existing studies on perinatal exposure to Mn reported both cognitive and behavioral effects[32].

Persistent organic pollutants

Persistent organic pollutants (POP) are contaminants highly remanent in environments and suspected of adverse effects on human health, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dioxins and organochlorine pesticides.
PCBs were widely used in electrical equipment as insulators and is currently banned in many countries. Developmental toxicity of PCBs was first described after exposure to high concentrations. There is less evidence about toxicity at low levels of exposure but literature reported adverse effects on neurodevelopmental and behavioral outcomes. Organochlorine compounds, including dioxins, hexachlorobenzene (HCB), organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT) were associated with poorer psychomotor development, cognitive skills and ADHD symptoms in literature.
Last, PBDEs are used as flame retardant additives. They are suspected to impair child neurodevelopment, in particular behavior, cognitive and motor skills[33].

Emerging neurotoxicant chemicals

Other chemical compounds are suspected to be neurotoxicant after exposure in-utero or during childhood. For example, phenols and phthalates have been associated with increased risk of autism spectrum disorders and cognitive or behavioral

31. Sharma et al., “An overview of worldwide and regional time trends in total mercury levels in human blood and breast milk from 1966 to 2015 and their associations with health effects”.
32. Sanders et al., “Perinatal and Childhood Exposure to Cadmium, Manganese, and Metal Mixtures and Effects on Cognition and Behavior”.
33. Grandjean and Landrigan, “Developmental neurotoxicity of industrial chemicals”; Grandjean and Landrigan, “Neurobehavioural effects of developmental toxicity”; Vrijheid et al., “Environmental pollutants and child health—A review of recent concerns”.

troubles[34].

In the same way, pesticide and solvent are suspected of neurotoxicity after prenatal exposure and are detailed in the next chapters[23].

34. Ejaredar et al., “Phthalate exposure and childrens neurodevelopment”; Mustieles et al., “Bisphenol A”; Ye et al., “The association of environmental toxicants and autism spectrum disorders in children”.

GLYCOL ETHERS: NEUROTOXICANT SOLVENTS?

3.1. Definition and properties

Solvent are liquids having a property to dissolve, dilute or extract other substances without any chemical modifications of those substances or themselves. There are thousands of solvents, some of them are used in daily life such as water. They are distinguished in two main classes: inorganic solvents without carbon atoms such as water (H_20) or ammonia (NH_3), and organic solvents. Organic solvents are further divided in chemical categories: petroleum (aliphatic or aromatic), oxygenated (alcohols, carboxylic acid, ketones, ethers, esters), halogenated (fluorinated, chlorinated, brominated, iodinated) and others.

GE are oxygenated solvents synthesized by reactions of alcohols and ethylene or propylene oxides. It gives two classes of GE: E-series (R-O-(CH2-CH2)$_n$-OH) for ethylene glycol derived and P-series (R-O-(CH2-CH(CH3))$_n$-OH) for propylene glycol derived. Their respective acetates are formed by reaction of carboxylic acid on GE. Two position isomers are formed during P-series synthesis: the major α-isomer, substituted on the first carbon atom from OH function and a minor β-isomer, substituted on the second carbon atom from hydroxyl-group (cf. Figure 3.1).

They have a low acute toxicity and are amphiphilic, i.e. hydrophilic and lipophilic. Their properties explain why they are widely used in domestic and occupational products, in particular in watery products. Production and sale of glycol ethers increased in the sixties together with the production of water paints[1].

GE also have a low boiling point, which results in an high vapor pressure and they are therefore considered as volatile organic compounds (VOC).

3.2. Uses in France

There are around eighty GE compounds and thirty of them are or have been used in France. E-series GE were more frequently used than P-series GE, with a ratio of 75/25 but the proportion tends to reverse (40/60 in 2006), since P-series compounds seem to be less toxicant[1].

The quantities used are constant over time, around 30,000 tons each year in France, and 60% (20,000 tons) are used as solvents, representing 4% of total solvent tonnage. Other uses are preservatives, antifreezes for aviation fuels, hydraulic fluids, synthesis intermediates, etc. In 2004, most used GE as solvents were propylene glycol methylether (PGME) (27%) and its acetate (48%), dipropylene glycol methylether (DPGME) (11%), diethylene glycol monobutylether (DEGBE) (7%), and propylene glycol ethylether (PGEE) (4%)[2].

1. Agence française de sécurité sanitaire de l'environnement et du travail (AFSSET), *Les Éthers de glycol*

2. Institut national de la santé et de la recherche médicale (France) (INSERM), *Éthers de glycol*; Institut national de recherche et de sécurité (INRS), *Les éthers de glycol*.

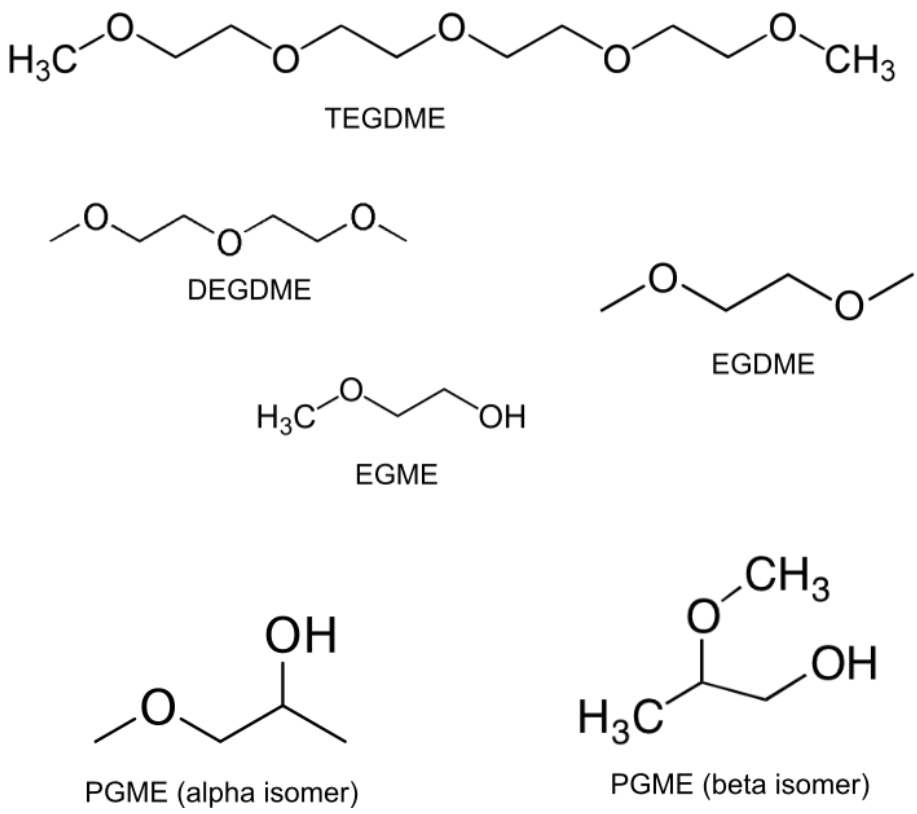


Figure 3.1 – Molecular formulas of some GE.

Considering all usages, most commercialized GE in 2006 were 1. PGME, 2. propylene glycol propylether (PGPE), 3. DPGME, 4. propylene glycol butylether (PGBE), 5. DEGBE, 6. dipropylene glycol n-butylether (DPGnBE) and 7. diethylene glycol ethylether (DEGEE)[1].

GE are found in various products, such as paints (aircraft, automobile, buildings), inks (printing, furniture), varnishes (furniture, car, metals), biocides, phytopharmaceutical products, household and industrial cleaning products (detergent, disinfectant, degreasers), cosmetic products (hair dyes, body products) and drugs (human and veterinary use). Exposures occur during both production (occupational exposure), use (occupational and domestic exposures or re-emission of furniture) of those products.

Occupational exposure is a common source of exposure. The Surveillance médicale des expositions des salariés aux risques professionnels (SUMER) survey carried out in France in 2010 reported that 13% of employees were exposed to solvents, including 1.6% to E-series GE and 1% to P-series GE. Workers in manufacturing plants, motor mechanics, maintenance, printing process and construction supply, hairdressers and beauticians, domestic helpers and cleaning workers were the most exposed to GE[3].

3. Vinck and Memmi, *Les expositions aux risques professionnels Les produits chimiques Enquête Sumer 2010*.

Women represent 50% of workers exposed to glycol ethers and child-bearing age groups (<25-39 years-old) are also likely to be occupationally exposed to GE. Therefore, pregnant women may be exposed to glycol ethers. More concerning, 3% of female workers are exposed to carcinogenic, mutagenic and reprotoxic (CMR) substances, 7600 women to CMR-classified GE[4].
People from the general population are also exposed after domestic uses of products containing GE. A German biomonitoring study on adults not exposed to GE at work detected several GE metabolites in more than a third of urine samples: 2-methoxypropionic acid (2-MPA) (34%), 2-phenoxypropionic acid (2-PhPA) and 2-butoxyethoxypropionic acid (2-BEAA) (41%), ethoxyacetic acid (EAA) (45%), butoxyacetic acid (BAA) (52%) and methoxyacetic acid (MAA) and phenoxyacetic acid (PhAA) in all urine samples[5]. Similarly, in the Perturbateurs Endocriniens : Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance (PELAGIE) cohort, Garlantézec *et al.* reported that methoxyethoxyacetic acid (MEAA), BAA and PhAA were found in urines of more than half of pregnant women in Brittany between 2002 and 2006 (56%, 93% and 93% respectively)[6].

Occupational exposure occurs mainly after cutaneous contact with GE during production or uses of products. The general population is likely to be exposed through multiple sources, mainly indoor environments (use of paints, cleaning products), cosmetic uses and drug ingestion. Cutaneous and respiratory ways are the main absorption ways, digestive absorption seems limited.

3.3. Regulations in France and UE

Due to their toxicity on human health and their volatility, the use of several GE has been restricted or banned since 1999 (cf. Table 3.1).
In 2015, for the E-series, eight GE were classified for reprotoxicity as category 1A (known) or 1B (presumed) under the Globally Harmonized System of classification and labelling of chemicals (GHS) and are marked with the Hazard Phrase H360 – "May damage fertility or the unborn child": ethylene glycol methylether (EGME), ethylene glycol methylether acetate (EGMEA), ethylene glycol dimethylether (EGDME), ethylene glycol ethylether (EGEE), ethylene glycol ethylether acetate (EGEEA), diethylene glycol dimethylether (DEGDME), triethylene glycol dimethylether (TEGDME), ethylene glycol diethylether (EGDEE). The diethylene glycol methylether (DEGME) is classified as category 2 (suspected) and is marked with the Hazard Phrase H361 – "Suspected of damaging fertility or the unborn child". The 1-propylene glycol 2-methylether (1PG2ME) and its acetate 1-propylene glycol 2-methylether acetate

4. Leonard et al., "Grossesse et travail".
5. Fromme et al., "Exposure of German residents to ethylene and propylene glycol ethers in general and after cleaning scenarios".
6. Garlantézec, Warembourg, et al., "Urinary Glycol Ether Metabolites in Women and Time to Pregnancy".

(1PG2MEA) are also classified for reproductive toxicity as category 1B under the GHS.

The use of DEGDME is specifically authorized by the European Registration, Evaluation, Authorization and restriction of CHemicals (REACH) regulation (annex XIV). All GE classified as reprotoxicant are registered on the annex XVII of REACH. Due to their volatile properties and toxicity after inhalation or cutaneous contact, DEGME and DEGBE are also restricted on annex XVII[7].

In France, ethylene glycol phenylether (EGPhE), ethylene glycol butylether (EGBE), DEGBE and DEGEE are restricted in cosmetics[1].

Reprotoxicant GE have been banned for use in consumer goods but are still used in industries when there is no substitute. According to AFSSET report in 2008, it concerns EGME, EGEE, EGDME, DEGDME for specific applications in chemical or pharmaceutical production. Nevertheless, the volume of these GE is decreasing and only 0.1% of employees are likely to be exposed to CMR-classified GE[1].

Table 3.1 – Regulations of GE compounds in France

Molecule	Reprotoxicant classification	Year of regulation[1]	Regulation status in France
EGME	1B	1999	Banned in drugs and cosmetics, restricted for consumer products
EGMEA	1B	1999	Banned in drugs and cosmetics, restricted for consumer products
EGEE	1B	1999	Banned in drugs and cosmetics, restricted for consumer products
EGEEA	1B	1999	Banned in drugs and cosmetics, restricted for consumer products
EGDME	1B	2003	Banned in cosmetics, restricted for consumer products
EGDEE	1A	2008	Restricted for consumer products
EGBE	-	2005	Restricted for cosmetics (4% in hair dyers)
EGPhE	-	2009	Restricted for cosmetics (1% as preservatives)
DEGME	2	2010	Restricted for consumer products[2]
DEGDME	1B	2003	Banned in cosmetics, restricted for consumer products
DEGEE	-	2005	Restricted for cosmetics (1.5% with purity exceeding 99.5% and less than 0.2% of ethylene glycol)

7. Institut national de l'environnement industriel et des risques (INERIS), *Ethers de glycol*

DEGBE	-	2005	Restricted for consumer products and cosmetics (9% in hair dyers)[2]
TEGDME	1B	2003	Banned in cosmetics, restricted for consumer products
1PG2ME[3]	1B	2004	Restricted for consumer products
1PG2MEA[3]	1B	2004	Restricted for consumer products

[1] If several regulation decisions, first one.

[2] Regulation due to toxicant effects after inhalation or cutaneous contact.

[3] B-isomer not commercialized, impurity up to 0.5% of its α-isomer.

3.4. Toxicology

Studies about how xenobiotics administered to an organism are modified by itself usually distinguish four main steps: absorption, distribution, metabolization and excretion.

Human organisms absorb GE mainly via the skin (after direct cutaneous contact or vapors) or by respiratory tracts (vapors). In the first minutes after their absorption GE acetates are hydrolyzed into GE in blood or mucous membranes.

Due to their amphiphilic properties, they are rapidly distributed to the whole body and high concentrations have been found in liver, kidneys and fatty tissues.

Metabolization processes then take place in the liver. Glymes, di or tri-substituted GE (e.g. DEGDME or TEGDME), are dealkylated into mono-substituted GE (i.e. EGDME) by monooxygenase cytochromes P450 (CYP450) and/or esterase. The further steps differ according to the chemical structure: E-series GE and β-isomers of P-series GE with a primary alcohol function are oxidized into aldehyde then carboxylic acids with the successive catalysations of alcohol-dehydrogenase and aldehyde-dehydrogenase enzymes. A-isomers of P-series cannot be turned into aldehydes (secondary alcohol function). They are metabolized into propylene glycol and alcohol by monooxygenase CYP450. Alcohol is ultimately metabolized in carbon dioxide (CO_2) (cf. Figure 3.2).

There is also a secondary metabolization pathway through monooxygenase CYP450 which turns GE in alcohol and ethylene- or propylene-glycol for E-series and P-series respectively, then in CO_2. This minor metabolic pathway is used in particular when and if alcohol-dehydrogenase and aldehyde-dehydrogenase enzymes are saturated[8].

Metabolic products of GE are: alkoxycarboxylic acids, ethylene or propylene glycol, CO_2 or unmetabolized GE. CO_2 is excreted via lungs. Ethylene glycol and propylene glycol are excreted in urine as unchanged compounds or conjugated to glucuronic or sulfonic acids. Alkoxycarboxylic acids and unmetabolized GE are found in urine

8. Institut national de la santé et de la recherche médicale (France) (INSERM), *Éthers de glycol*

where unmetabolized forms represent less than 10% of urinary excretion. Chemical structure of alkoxycarboxylic acids depends of their parent compounds (cf. Table 3.2).

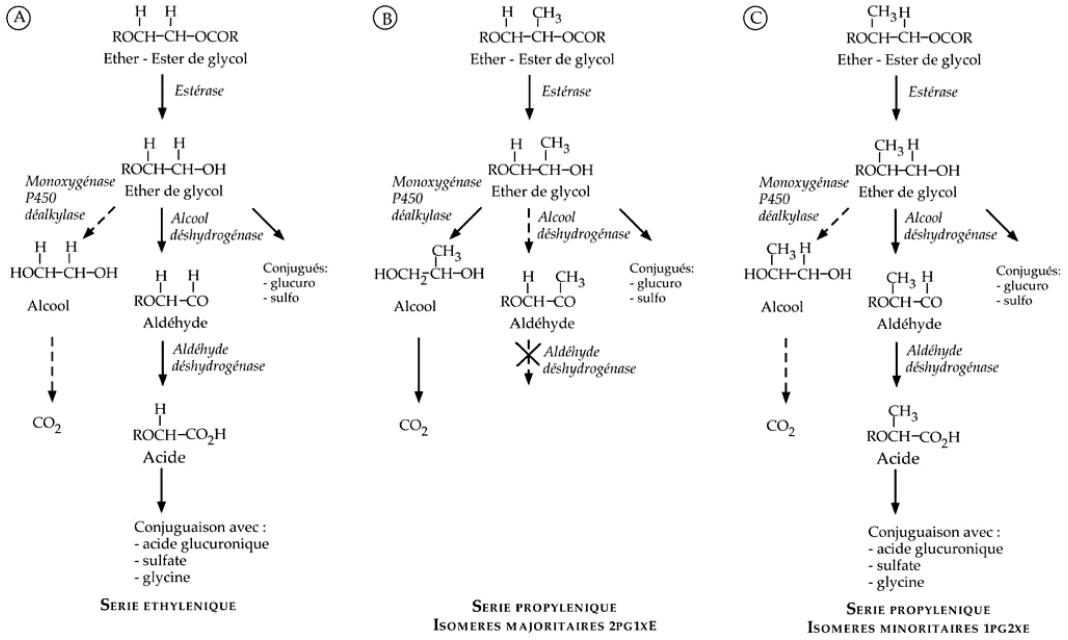


Figure 3.2 – Metabolism of glycol ethers[8].

Plasmatic half-life of GE is around 20-30 min. Alkoxycarboxylic acids are responsible for toxicant effects of GE and their half-life, depending on the length of their alkyl chain, is up to 80 hours. Some alkoxycarboxylic acids have known hematotoxicant properties, in particular BAA causing hemolysis effects (alteration of erythrocyte plasticity, modifications of shape or volume increase), bone marrow hypoplasia effects and immunotoxicity of MAA and its aldehyde precursor (methoxyacetaldehyde). Foetotoxicant effects of MAA may result of abnormal apoptosis during spermatogenesis (reprotoxicity) or at specific time periods of development (teratogenicity).

Table 3.2 – Alkoxycarboxylic acids produced by metabolization of GE

Parent compounds (glycol ethers)	Metabolites
MAA	EGME, EGDME, DEGME, DEGDME, TEGME, TEGDME
EAA	EGEE, EGDEE, DEGEE, DEGDEE, TEGEE
BAA	EGBE, DEGBE, TEGBE
PAA	EGiPE, EGnPE
PhAA	EGPhE
MEAA	DEGME, TEGME
EEAA	DEGEE, TEGEE
BEAA	DEGBE, TEGBE

2-MPA	1PG2ME
2-EPA	1PG2EE

3.5. Developmental neurotoxicant effects

Regulation decisions about GE are mostly based on their reprotoxicant properties, including fertility, foetotoxicy and teratogenicity. Corpus of studies also evaluated their potential hematoxicity but there is, at our knowledge, very few studies about their neurotoxicity on brain.
Neurological effects of GE are described after accidental exposure to high levels: somnolence, headache, dizziness, coma[9].
Only a few animal studies carried out in the eighties investigated neurotoxicant effects of GE after prenatal exposure. Nelson *et al.* exposed pregnant Sprague-Dawley rats exposed via inhalation to 25 parts-per-million (ppm) of EGME or to 100 ppm of EGEE during gestation days 7-13 or 14-20. Doses of GE showed no effects on female rats, except a slightly prolonged gestation in the group exposed to 100 ppm EGEE on days 14-20. Compared to controls, rats exposed in-utero to EGME or EGEE performed poorly to the learning test and present neurochemistry alterations, in particular in the brainstem and cerebrum[10].

Most epidemiological studies investigated neurodevelopment of children in association with exposure to solvents in general, and in most cases in a context of maternal occupational exposure (cf. Table 3.5).
A study comparing exposed versus unexposed children from a US cohort found no association between maternal occupational exposure during pregnancy and child's behavior at 3.5 years of age and a slightly earlier age at walking (δ = 0.8 months)[11].
In Canada, women were recruited from the prospective Motherisk program between 1992 and 1996. This hospital service advised women during their pregnancy about exposure to organic solvents or nonteratogens. An exposure index was built from the parameters of their occupational exposures to solvents (length and frequency of exposure, symptoms, individual and collective protections). They reported associations between increased exposure and lower scores of language and graphomotor abilities but no association with attention, visuospatial, fine-motor scores in three to seven year-old children. There were no differences of behavioral scores but exposed children were more likely to present mild or severe internalizing or externalizing be-

9. Institut national de recherche et de sécurité (INRS), *Les éthers de glycol*.
10. Nelson and Brightwell, "Behavioral teratology of ethylene glycol monomethyl and monoethyl ethers."; Nelson, Brightwell, et al., "Reproductive toxicity of the industrial solvent 2-ethoxyethanol in rats and interactive effects of ethanol."
11. Eskenazi, Gaylord, et al., "In Utero Exposure to Organic Solvents and Human Neurodevelopment"

havioral disorders[12]. In 2004, a similar study from the Motherisk program compared neurodevelopment of exposed children to controls. At age 3-9 years, children were tested for cognitive functioning (IQ), language, visual-motor functioning, and behavioral functioning. Exposed children had lower scores of intellectual, language, motor, and neurobehavioral functioning[13]. Then, still from the Motherisk program, Till *et al.* reported reduced alterations of vision in infants prenatally exposed to solvents[14,15]. In 2013, Pelé *et al.* investigated effects of occupational exposure to solvents during pregnancy and child behavior at two years of age in the PELAGIE population-based cohort. Children whose mothers were exposed at work to products containing solvents presented higher scores of attention deficit/hyperactivity and aggression with a dose-response relationship shape[16]. Considering externalizing and internalizing behavior at both age 2 and 6 years old in the PELAGIE cohort, authors found higher scores of externalizing behavior disorders in 2 year-old children exposed in-utero to solvents. This association was still found at age 6 but attenuated and not statistically significant[17].

To our knowledge, there is only one existing epidemiological study investigating specific effects of prenatal exposure to GE on child's neurodevelopment. In the PELAGIE cohort, child's cognition was assessed by the administration of the Wechsler Intelligence Scale for Children (WISC) and NEPSY tests. Children with high maternal levels of PhAA and EAA had lower scores of verbal comprehension and copying scores, respectively[18].

12. Till, Koren, et al., "Prenatal exposure to organic solvents and child neurobehavioral performance"

13. Laslo-Baker et al., "Child Neurodevelopmental Outcome and Maternal Occupational Exposure to Solvents"

14. Till, C. A. Westall, et al., "Effects of maternal occupational exposure to organic solvents on offspring visual functioning"

15. Till, C. Westall, et al., "Vision abnormalities in young children exposed prenatally to organic solvents"

16. Pelé et al., "Occupational solvent exposure during pregnancy and child behaviour at age 2"

17. Costet et al., "Occupational exposure to organic solvents during pregnancy and childhood behavior"

18. Béranger et al., "Prenatal Exposure to Glycol Ethers and Neurocognitive Abilities in 6-Year-Old Children"

Table 3.3 – Studies about prenatal exposure to solvents and child's neurodevelopment

Paper	Study	N	Exposure assessment	Outcome	Results
Animal studies about GE					
Nelson *et al.* 1984 [10]	animal study (rats)	n=8	Inhalation to 25 ppm of EGME or to 100 ppm of EGEE on gestation days 7 to 13 or 14 to 20.	Behavioral testing (neuromuscular ability, activity, and learning ability) and brain concentrations of neurotransmitters (acetylcholine, dopamine, norepinephrine, and serotonine).	Poorer performances at avoidance conditioning test for rats exposed to EGME or EGEE during gestional days 7-13. Numerous variations of concentrations of neurotransmitters.
Epidemiological studies about solvents					
Eskenazi *et al.* 1988 [11]	Exposed vs. non-exposed in New Heaven area (USA) (1980-1982)	n=41/41	Industrial hygienist review of maternal job description (questionnaire at $\pm$ 3 months of pregnancy), organic solvents considered: aromatic, halogenated, ketones, aldehydes or miscellaneous.	Anthropometric measures (height, weight, head circumference), McCarthy Scales of Children's Abilities, mothers' reports of developmental milestones, Conners Parent Scale of Hyperactivity and The National Institute of Mental Health Childhood Personality Scale-Revised.	No association between exposure and anthropometric measures, developmental milestones, behavior or personality.

Continued on next page

Paper	Study	N	Exposure assessment	Outcome	Results
Till *et al.* 2001 [12]	Exposed vs. non-exposed in Toronto (Canada) (1992-1996)	n=33/28	Exposure index for occupational exposure during pregnancy based on questionnaire (length and frequency of exposure, symptoms, individual and collective protections).	NEPSY subtests, Wide Ranfe Assessment of Visual Motor Abilities subtests, Peabody Picture Vocabulary Test, Expressive One-Word Picture Vocabulary Test, visual CPT and Child Behavior Checklist (CBCL).	Negative associations between exposure and scores of Receptive language, Expressive language), and Graphomotor. No differences on measures of Attention, Visuospatial ability and Fine-motor ability. On the CBCL, no mean differences but exposed children were rated as having mild or severe problem behaviors.
Laslo-Baker *et al.* 2004 [13]	Exposed vs. non-exposed in Toronto (Canada) (1989-1998)	n=32/32	Questionnaire about occupational exposure during pregnancy (length and frequence of exposure, symptoms, individual and collective protections).	Wechsler Preschool and Primary Scale of Intelligence (3-6 yo) or Wechsler Intelligence Scale for Children (6-9 yo), Preschool Language Scale (3-6 yo) or Clinical Evaluation of Language Fundamentals (6-9 yo), Beery-Buktenica Developmental Test of Visual-Motor Integration, Grooved Pegboard Test, Child Behavior Checklist (CBL), Conner's Rating Scale–Revised, Behavioral Style Questionnaire. Maternal cognitive functioning was measured by using the Wechsler Abbreviated Scale of Intelligence.	Negative associations between exposure and scores of Digit span, information, vocabulary, and recalling sentences, grooved pegboard and Conner's hyperactivity/ impulsivity diagnostic.

Continued on next page

Paper	Study	N	Exposure assessment	Outcome	Results
Till *et al.* 2001 [14]	Exposed vs. non-exposed in Toronto (Canada) (1992-1996)	n=33/28	Exposure index for occupational exposure during pregnancy based on questionnaire (length and frequence of exposure, symptoms, individual and collective protections).	Minimalist Test and Cardiff Cards for monocular and binocular vision in 3-7 yo.	Positive associations between exposure and error scores on red-green and blue-yellow color discrimination and poorer visual acuity. No dose-response relationship.
Till *et al.* 2005 [15]	Exposed vs. non-exposed in Toronto (Canada) (1999-2003)	n=21/27	Questionnaire about occupational exposure during pregnancy (length and frequence of exposure, symptoms, individual and collective protections).	Contrast sensitivity and grating acuity using a sweep visual evoked potential (VEP) technique in 6-25 mo.	Association between exposure and reduction in grating acuity and contrast sensitivity in the low and intermediate spatial frequency range. Exposed children produced abnormal VEP responses to the red-green onset stimulus, but not to either blue-yellow or achromatic stimuli. No differences of latency or amplitude of chromatic and achromatic response.
Pelé *et al.* 2013 [16]	PELAGIE mother-child cohort in Brittany, France (2002-2006)	n=1,278	Questionnaire about frequency of occupational exposure to 11 products containing solvents and classification as not exposed (no exposure to any of products), occasionally exposed (occasional exposure to at least one product) and regularly exposed (regular exposure to at least one product).	Quebec Longitudinal Study of Child Development (QLSCD) based on Child Behavior Checklist and the Preschool Social Behavior Questionnaire.	Dose-response relationship between exposure and attention deficit/hyperactivity ans aggression scores.

Continued on next page

Paper	Study	N	Exposure assessment	Outcome	Results
Costet *et al.* 2018 [17]	PELAGIE mother-child cohort in Brittany, France (2002-2006)	n=715	Questionnaire about frequency of occupational exposure to 11 products containing solvents and classification as not exposed (no exposure to any of products), occasionally exposed (occasional exposure to at least one product) and regularly exposed (regular exposure to at least one product).	Child Behavior Checklist and the Preschool Social Behavior Questionnaire (2 yo) and the Strength and Difficulties Questionnaire (6 yo).	Positive association between exposure and externalizing behavior disorder score at age 2, but attenuated at age 6 years. No association for internalizing behavior.
Béranger *et al.* 2017 [18]	PELAGIE mother-child cohort in Brittany, France (2002-2006)	n=204	Five urinary metabolites (MAA, EAA, EEAA, BAA and PhAA) collected <19 WG.	WISC and NEPSY tests administered at 6 yo.	Association between high urinary levels of PhAA and WISC Verbal Comprehension Score and between EAA and NEPSY Design Copying subtest score. No association with other GE metabolites.

ORGANOPHOSPHATES, NEUROTOXICANT INSECTICIDES

4.1. Definition and properties

The term "pesticide" usually refers to products used in agriculture for crop treatments. However, this term encompasses all substances used to control pest, in plants (i.e. plant protection products), and humans or animals (biocides).
In 2019, the European commission authorized 476 active substances for phytopharmaceutical uses[1]. These substances can be divided across their uses (such as herbicides, fungicides or insecticides) or their chemical functions (such as carbamates, pyrethroids, organophosphates, organochlorines, and neonicotinoids for insecticides purposes).
Organophosphates (OP) were commercialized worldwide after the World War II for agricultural and pest control purposes. Their uses expanded after the seventies when most organochlorine were restricted or banned due to persistence in environment and concerns about human health. OP are also more efficient than organochlorine due to their high acute toxicity, for example organochlorine DDT has a median lethal dose (LD50) of 113-800 mg/kg in rats after a single oral dose whereas LD50 of chlorpyrifos is 7.9-14 mg/kg in the same conditions[2].
OPs are a large class of pentavalent phosphorus acid ester, with general molecular formula (R_1O, R_2O) – PO (or S) – O (or S) – X. They are further divided according to the nature of the X group in 3 classes (cf. Figure 4.1):

- Aliphatic OP (e.g. malathion) have a high acute toxicity but are unstable.
- Aromatic OP (e.g. parathion) are more persistent.
- Heterocyclic OP (e.g. phosmet).

They can also be divided whether or not they have a sulfur atom in: organophosphates (e.g. dichlorvos), thiophosphates (e.g. chlorpyrifos) or dithioorganophosphates (e.g. malathion). Most OP are slightly soluble in water and have a low volatility, except dichlorvos.

4.2. Uses and exposure in France

There are sixty-five OP active substances currently registered in ANSES E-phy database but only eight of them (chlorpyrifos-ethyl, chlorpyrifos-methyl, fenamiphos, fosthiazate, malathion, phosmet, pirimiphos-methyl and tolclofos-methyl) are currently authorized for plant protection in France (cf. Appendix on page 197). The ANSES database contains information about phytopharmaceutical products in France, their uses and regulations. It also provides with specific information on active substances, product adjuvants and mixtures.

1. European Commission, *EU Pesticides database*
2. Agency for Toxic Substances and Disease Registry (ATSDR), *Toxicological Profiles*.

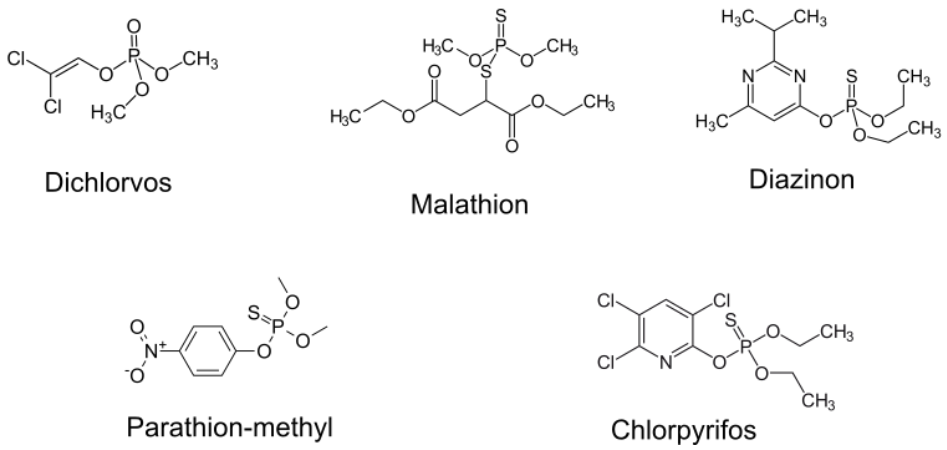


Figure 4.1 – Molecular formulas of OP insecticides.

Most of OP authorizations have been removed by the European commission in 2002[1]. Meanwhile they are more and more replaced by neonicotinoid and pyrethroid compounds which are assumed to be less toxicant for mammalians. Organophosphates still represent a large rate of insecticide sales in France (on 3,700 tons of insecticides sold in 2017, 290 tons were organophosphates and 270 tons carbamates)[3].
In 2010 in France, OP compounds were found in 35% of veterinary anti-parasitic, in particular collars (84.3%). The most commonly found OP were coumaphos, dichlorvos, diazinon, fenitrothion, fenthion, propetamphos and tetrachlorvinphos. Malathion use was also authorized for anti-lice human treatment[4].
OP were found in 39% of house airs, in particular diazinon (20%) and dichlorvos (14%). Passive aeration, use of anti-lice products, number of plants and no garden were associated with increased levels of OP in indoor air. Dichlorvos (11%), fenthion (15%), malathion (23%), methyl-parathion (14%) and diazinon (26%) were also detected on indoor soils[5] .

In years 2000-2010, exposure of the general population occurred mainly after accidental inhalation or cutaneous contact resulting from domestic uses and presence of pesticide residues in diets. Nowadays, it seems likely that the general population is mostly exposed through dietary residues since the use of OP has decreased in domestic products. For occupational exposure, the cutaneous way prevails during preparation or application of pesticides[6].

3. Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), *E-phy database*; European Commission, *Pesticide sales*; Schleier and Peterson, "CHAPTER 3".

4. Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), *Exposition de la population générale aux résidus de pesticides en France: Synthèse et recommandations du comité d'orientation et de prospective scientifique de l'observatoire des résidus de pesticides, ORP*.

5. Institut national de la santé et de la recherche (INSERM), *Pesticides - Effets Sur La Santé.*

6. Carrier et al., "Évaluation de l'exposition humaine aux organophosphorés et des risques associés à l'aide de biomarqueurs urinaires"; Institut national de la santé et de la recherche (INSERM),

In the 2000's, French pregnant women have frequently been exposed to OP. In the French mother-child cohort PELAGIE (Brittany, from 2002), pesticide residues were detected in most maternal urines (44 quantified molecules found from 1 to 84% of samples) and OP were the most frequently detected molecules[5].

4.3. Toxicology

4.3.1. Toxicokinetics

OP are absorbed through skin, respiratory or gastrointestinal tracts, depending on their formulation and use context.
They join the systemic system and are distributed rapidly to the whole organism with a plasmatic half-life of a couple of hours. Due to their instability, prolonged storage in human tissue is not expected. However, some OP are very lipophilic, such as chlorpyrifos or parathion, and may be stored into fatty tissues for several days.
Metabolism occurs principally by oxidation, and hydrolysis by esterases and by reaction with glutathione. Oxidation of OP pesticides may result in more or less toxic products. In general, thiophosphates, with a P = S bond, are not directly toxic but require oxidative metabolism to lead to an oxon with a P = O bond. This oxidative desulfurization mostly occurs in the liver, by CYP450.
The oxon can be enzymatically or spontaneously hydrolyzed to form a dialkylphosphate (DAP) metabolite and a specific metabolite moiety[7]. Catalytic hydrolysis of the OPs into DAP by phosphotriesterases known as α-esterases (which are not inhibited by OPs) plays an important role in the detoxification of certain OPs. One example is the enzyme paraoxonase 1 (PON1) which hydrolyzes the oxons of chlorpyrifos and diazinon.
According to the chemical structure of the OP, methyl or ethyl-substituted and presence or not of a sulfur atom, there are six types of DAP: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP) and diethyldithiophosphate (DEDTP), that may be grouped in dimethylphosphate (DM) and diethylphosphate (DE) metabolites. Elimination of the OP metabolites occurs mainly via the urine, or feces.

4.3.2. Modes of action

The main target for OP is AChE, a β-esterase whose physiological role is to hydrolyze ACh. OPs phosphorylate a hydroxyl group on serine amino acid in the active site of the enzyme, preventing its action on the physiological substrate. Phosphory-

Pesticides - Effets Sur La Santé. Muñoz-Quezada et al., "Neurodevelopmental effects in children associated with exposure to organophosphate pesticides"; Nougadère et al., "Total diet study on pesticide residues in France"; Raffy et al., "Semi-volatile organic compounds in the air and dust of 30 French schools".

7. Wessels et al., "Use of biomarkers to indicate exposure of children to organophosphate pesticides".

lated AChE is hydrolyzed by water at a very slow rate (from several hours to days). Reactivation of phosphorylated AChE does not occur once the enzyme-inhibitor complex has "aged," i.e., the loss by hydrolysis of one of the two alkyl groups (R_1 or R_2). When phosphorylated AChE has aged, the enzyme can be considered to be irreversibly inhibited, and the only way of replacing its activity is through synthesis of new enzyme, a process that may take days.
Inhibition of AChE by OPs causes accumulation of ACh at cholinergic synapses, with overstimulation of cholinergic receptors of the muscarinic and nicotinic types. As these receptors are localized in most organs of the body, a "cholinergic syndrome" ensues, which includes increased sweating and salivation, profound bronchial secretion, bronchoconstriction, miosis, increased gastrointestinal motility, diarrhea, tremors, muscular twitching, and various CNS effects (dizziness, inhibition of central respiratory centers, convulsions, coma).
OP may also inhibit butylcholinesterases as well as other esterases. The effects of butylcholinesterase inhibition are unknown, but its inhibition can provide an indication of exposure to OPs.

The possibility that OPs may act on other targets is still debated. For example, developmental neurotoxicity observed at doses that cause no or a minimal AChE inhibition suggests that other biological systems may be affected by OPs.
OPs have been shown to interact with nicotinic receptors at high concentrations and at lower concentrations (nanomolar range) with muscarinic receptors. Other pre- and post-synaptic components of the cholinergic system have also been reported to be affected by OPs, such as choline acetyltransferase and different kinases involved in signal-transduction.
Several enzymes involved in the metabolism of peptides are inhibited by OPs. For example, acylpeptide hydrolase (APH), responsible for the removal of N-acetylated amino acids from the N-terminus of short peptides, such as beta-endorphin, is inhibited by various oxons (e.g., dichlorvos, diazoxon) at low concentrations, and inhibition was also observed after *in-vivo* exposure. OPs can also target the cannabinoid system, though its toxicological significance remains unclear. Two potentially important noncholinergic effects of OPs that have been investigated are oxidative stress and neuroinflammation[8].
OP also affect neuronal metabolism at low-dose, such as alteration of axonal transport or mitochondria metabolism, loss of neurons and glial cells or persistent serotonergic effects[9].

8. Costa, "Current issues in organophosphate toxicology".
9. Chen et al., "Mother gestational exposure to organophosphorus pesticide induces neuron and glia loss in daughter adult brain"; Slotkin and Seidler, "The alterations in CNS serotonergic mechanisms caused by neonatal chlorpyrifos exposure are permanent"; Terry, "Functional consequences of repeated organophosphate exposure".

4.4. Neurotoxicant effects

There is a large body of evidence about acute effects of OP on brain function but findings of neurotoxicant effects after exposure at low doses are less consistent, in particular during neurodevelopmental periods (cf. Table 4.1).
Several epidemiological studies have investigated potential adverse neurodevelopmental effects after prenatal exposure. Three recent reviews have drawn up a state of the art of the main findings.
Koureas *et al.* reviewed all biomonitoring studies about organophosphate and pyrethroid exposures and human health outcomes between 1991 and 2012. US cohort studies found consistent associations between prenatal OP exposure and abnormal reflexes in neonates. The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, a cohort started in 1999 enrolling Mexican-American pregnant women living in agricultural communities of the California's Salinas Valley, reported inconsistent findings with cognitive development at 6, 12 and 24 months of age but suggested attention deficit in 3.5 and 5 year-old children and poorer intellectual development in 7 year-old children. It is consistent with the Mount Sinai study results of delayed intellectual development at age 12 months and slight decrement in 6 to 9 year-old children. The Mount Sinai study is a multiethnic cohort in New York City that enrolled primiparous women who presented for prenatal care with singleton pregnancies at the Mount Sinai prenatal clinic and two private practices, and were delivered at Mount Sinai Hospital between May 1998 and July 2001. The Columbia Center for Children's Environmental Health (CCCEH) in New York City recruited non-smoker Dominican and African-American women residing in Washington Heights, Central Harlem, and the South Bronx. They reported mental and motor delay, attention and ADHD disorders in association with prenatal chlorpyrifos exposure at 12, 24 and 36 months of age. A cross-sectional study in Ohio and Mississippi found alterations in short-term memory, attention, behavior and motor functions in 6 year-old children exposed to methyl-parathion, but these results were not consistent between the two sites. In Ecuador, 6-9 year-old children presented increased response latency and attention alterations in association with childhood exposure to OP. A study of National Health and Nutrition Examination Survey (NHANES) suggested increased ADHD troubles at 8-15 years of age[10].
A review of 27 studies on neurodevelopmental effects of OP exposure reported that 10 of them suggested cognitive (working memory), behavioral (attention) or motor (reflexes) deficits. Eleven (on twelve) studies found dose-response relationship. Nevertheless, they pointed out the diversity of type of exposure or outcome measurements, making it difficult to do a meta-analysis and enforcing the need to find

10. Koureas et al., "Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes"

the critical window of development[11].

González-Alzaga *et al.* reviewed all papers until December 2012 investigating prenatal and postnatal neurodevelopmental effects of OP exposure. According to them, prenatal exposure to OP is associated with mental development delays, more inconsistently with psychomotor development, attention problems and ADHD disorders. They suggested possible non-cholinergic mechanisms (decreased number of neurites, alteration of the differentiation of C6 glioma cells) and genetic susceptibility and addressed the necessity to investigate possible sex specific effects, due to genetic or social construction[12].

Since 2012, US cohorts have pursued the neurodevelopmental follow-up of included children. In the CHAMACOS cohort, higher cumulative pesticide use were associated with deficits in IQ score in 7-year-old children[13,14]. Residential proximity to cultures was also associated with poor cognitive functioning at 10 years of age[15]. Then a recent pilot study to be published using fNIRS technique on 95 CHAMACOS adolescents found associations between prenatal exposure to OP and altered brain activation patterns during tasks of language comprehension and executive functions (working memory, cognitive flexibility yet not for response inhibition) but no major differential performances[16].

The CCCEH study found no significant remediating effect of home environment following prenatal exposure to chlorpyrifos but borderline significant effect of child sex (bigger decrement in boys), suggesting that boys may benefit more of nurturing environment than girls[17]. They also reported deficit in working memory and full scale IQ at 7 years of age and increased tremor in 11 year-old children prenatally exposed to chlorpyrifos[18,19]. Moreover, Rauh *et al.* found sex-specific alterations of the brain structure in children exposed to chlorpyrifos and an interaction between these alterations and IQ scores[20].

11. Muñoz-Quezada et al., "Neurodevelopmental effects in children associated with exposure to organophosphate pesticides"
12. González-Alzaga et al., "A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure"
13. Coker et al., "Association between Pesticide Profiles Used on Agricultural Fields near Maternal Residences during Pregnancy and IQ at Age 7 Years"
14. Gunier et al., "Prenatal Residential Proximity to Agricultural Pesticide Use and IQ in 7-Year-Old Children"
15. Rowe et al., "Residential proximity to organophosphate and carbamate pesticide use during pregnancy, poverty during childhood, and cognitive functioning in 10-year-old children"
16. Sagiv et al., "Prenatal exposure to organophosphate pesticides and functional neuroimaging in adolescents living in proximity to pesticide application"
17. Horton et al., "Does the home environment and the sex of the child modify the adverse effects of prenatal exposure to chlorpyrifos on child working memory?"
18. V. Rauh et al., "Seven-Year Neurodevelopmental Scores and Prenatal Exposure to Chlorpyrifos, a Common Agricultural Pesticide"
19. V. A. Rauh, Garcia, et al., "Prenatal exposure to the organophosphate pesticide chlorpyrifos and childhood tremor"
20. V. A. Rauh, Perera, et al., "Brain anomalies in children exposed prenatally to a common organophosphate pesticide"

The Mount Sinai cohort suggested poorer social functioning in boys and black people after prenatal exposure to OP. They also found negative association between DE levels and working memory index and DM levels and internalizing factor scores but a positive association between DM concentrations and executive functioning[21,22].

The Health Outcomes and Measures of the Environment Study (HOME Study) is an ongoing prospective pregnancy and birth cohort study from 2003 in Cincinnati. Authors reported no association between prenatal exposure to OP and cognition at ages 1 to 5 years, nor social behavior at 8 years of age, nor susceptibility modification with the PON1 allele status. They suggested a possible protection of socioeconomic status and healthier diet[23,24].

In Asia, recent cohort studies also assess the association between prenatal exposure to OP and neurodevelopmental outcomes. In China, the Shangong cohort reported association at 24 months yet not at 12 months of age between DAP and DE levels and social domains and, in boys only, with developmental quotients. They found opposite associations with postnatal exposure[25]. The Shenyang cohort suggested inverse association between OP exposure and scores of neurobehavioral development of neonates[26]. The Funyang cohort found deficits in motor function associated with chlorpyrifos and naled (insecticides used against Zika mosquitoes) exposure and these deficits were more pronounced in girls. There were also deficits in vision and reduced head circumference at birth associated with chlorpyrifos levels in 6-week to 18-month-children[27,28]. Then, total DAP levels were associated with reduced cognitive composite scores in 5-month-children from a birth cohort in Thailand[29].

In Europe, the PELAGIE mother-child cohort reported association between prenatal urinary levels of DE in French pregnant women and decreased contrast sensitivity in boys only, and non-monotonous relation between DM level and increased score

21. Furlong, Engel, et al., “Prenatal exposure to organophosphate pesticides and reciprocal social behavior in childhood”
22. Furlong, Herring, et al., “Prenatal exposure to organophosphorus pesticides and childhood neurodevelopmental phenotypes”
23. Donauer et al., “An Observational Study to Evaluate Associations Between Low-Level Gestational Exposure to Organophosphate Pesticides and Cognition During Early Childhood”
24. Millenson et al., “Urinary organophosphate insecticide metabolite concentrations during pregnancy and children’s interpersonal, communication, repetitive, and stereotypic behaviors at 8 years of age”
25. Wang et al., “Prenatal and postnatal exposure to organophosphate pesticides and childhood neurodevelopment in Shandong, China”
26. Zhang et al., “Prenatal Exposure to Organophosphate Pesticides and Neurobehavioral Development of Neonates”
27. Silver, Shao, Zhu, et al., “Prenatal naled and chlorpyrifos exposure is associated with deficits in infant motor function in a cohort of Chinese infants”
28. Silver, Shao, Ji, et al., “Prenatal organophosphate insecticide exposure and infant sensory function”
29. Kongtip et al., “The Impact of Prenatal Organophosphate Pesticide Exposures on Thai Infant Neurodevelopment”

in girls. No association was found between prenatal exposure to OP and cognitive function at 6 years of age, but children with higher urinary levels of DE at 6 years presented lower working memory scores[30,31].

Recently, the Generation R in the Netherlands reported small and inconsistent associations between DAP concentrations in late pregnancy (>25 weeks) and lower IQ scores, and no heterogeneity effect with PON1 allele status. They suggested a vulnerability window for neurotoxicant effects in late pregnancy and a protective factor status of the consumption of fruits and vegetables[32].

30. Cartier, Warembourg, Le Maner-Idrissi, et al., “Organophosphate Insecticide Metabolites in Prenatal and Childhood Urine Samples and Intelligence Scores at 6 Years of Age”

31. Cartier, Warembourg, Monfort, et al., “Children’s contrast sensitivity function in relation to organophosphate insecticide prenatal exposure in the mother-child PELAGIE cohort”

32. Jusko et al., “Organophosphate Pesticide Metabolite Concentrations in Urine during Pregnancy and Offspring Nonverbal IQ at Age 6 Years”

Table 4.1 – Epidemiological studies about prenatal exposure to OP and child's neurodevelopment

Paper	Study	N	Exposure assessment	Outcome	Results
Review until 2012					
Koureas *et al.* 2012 [10]	1991-2012	n=49 studies	Urinary or plasmatic biomarkers of OP and pyrethroids.	Health outcomes.	Associations between prenatal exposure to OP and reflexes in neonates, delays of intellectual development and attention troubles. Points out child's brain vulnerability because of its fast development.
Munoz-Quezada *et al.* 2013 [11]	2002-2012	n=27 studies	Exposure to OP.	Neurodevelopmental effects.	Associations with cognitive deficits (working memory), behavioral (attention) or motor (reflexes) troubles, with dose-response relationship. Highlights the diversity of measurements of exposure or outcomes (no meta-analysis) and the need to find the critical window of development.
Gonzalez-Alzaga *et al.* 2014 [12]	Until December 2012	n=20 studies	Prenatal and postnatal exposure OP.	Neurodevelopmental effects.	Prenatal exposure to OP associated with mental development delays, more inconsistently with psychomotor development and attention problems. Suggests possible non-cholinergic mechanisms and genetic susceptibility. Addresses the necessity to investigate possible sex specific effects (genetically or by social construction).
Studies published after 2012					

Continued on next page

Paper	Study	N	Exposure assessment	Outcome	Results
Coker *et al.* 2017 [13]	CHAMACOS mother-child cohort (Salinas Valley, California - 1999-2000)	n=255	Residential proximity to culture during pregnancy and PUR data.	Full-Scale Intelligence Quotient (FSIQ) at 7 years of age.	Highest cumulative pesticide use levels were associated with deficits in adjusted FSIQ of -6.9.
Gunier *et al.* 2017 [14]	CHAMACOS mother-child cohort (Salinas Valley, California - 1999-2000)	n=283	Residential proximity to culture during pregnancy and PUR data.	WISC-IV at 7 years of age.	Decreased IQ scores with OP pesticide uses, in particular acephate and oxymeton-methyl.
Rowe *et al.* 2016 [15]	CHAMACOS mother-child cohort (Salinas Valley, California - 1999-2000)	n=501	Residential proximity to culture during pregnancy and PUR data.	WISC-IV score at 10 yo.	Residential proximity to OP associated with poor cognitive functioning at 10 years of age.
Sagiv *et al.* 2019 [16]	CHAMACOS mother-child cohort (Salinas Valley, California - 1999-2000)	n=95	Residential proximity to culture during pregnancy and PUR data.	fNIRS intensities during tasks of executive function (Go/No-Go task, Wisconsin Card Sorting Test, Visuospatial N-back working memory task and Sternberg working memory task), language comprehension (Pyramids and Palm Trees) and social cognition (Dynamic social gesture task).	Association between exposure to OP and brain activation patterns during tasks of language comprehension and executive function (working memory, cognitive flexibility). No major alteration of task performance.
Horton *et al.* 2012 [17]	CCCEH mother-child cohort (New York City, 1999-2002)	n=335	Cord blood levels of chlorpyrifos at delivery.	Working memory scale of WISC at 7 years old.	No statistically significant remediating effect of home environment but borderline significant effect of child sex (effect in boys +++).

Continued on next page

Paper	Study	N	Exposure assessment	Outcome	Results
Rauh *et al.* 2011 [18]	CCCEH mother-child cohort (New York City, 1999-2002)	n=265	Cord blood levels of chlorpyrifos at delivery.	WISC-IV at 7 years old.	Deficit in working memory and full scale IQ at 7 years of age.
Rauh *et al.* 2015 [19]	CCCEH mother-child cohort (New York City, 1999-2002)	n=263	Cord blood levels of chlorpyrifos at delivery.	Archimed spirals at 11 years of age.	Increased tremor with dominant arm, almost statistically significant with non-dominant arm.
Rauh *et al.* 2012 [20]	CCCEH mother-child cohort (New York City, 1999-2002)	n=2*20	Cord blood levels of chlorpyrifos at delivery.	MRI T1-weighted anatomical sequence at 7-8 years of age and WISC-IV.	Higher exposure associated with enlargement of superior temporal, posterior middle temporal, and inferior postcentral gyri bilaterally, enlarged superior frontal gyrus, gyrus rectus, cuneus, and precuneus along the mesial wall of the right hemisphere, and frontal and parietal cortical thinning. Significant IQ*exposure interaction and sex × exposure interaction within the right inferior parietal lobule, right superior marginal gyrus, and right mesial superior frontal gyrus.
Furlong *et al.* 2014 [21]	Mount Sinai mother-child cohort (New York City - 1998-2001)	n=136	Maternal urinary DAP (25-40 WG).	Social Responsiveness Scale (7-9 years old).	Associations between DAP levels and poorer social functioning, in boys and black people.
Furlong *et al.* 2017 [22]	Mount Sinai mother-child cohort (New York City - 1998-2001)	n=404	Maternal urinary DAP (25-40 WG).	Behavior Rating Inventory of Executive Functioning (BRIEF) in 6-9 yo.	DM levels negatively associated with internalizing factor scores and positively with executive functioning. DE levels associated with poorer working memory index.

Continued on next page

Paper	Study	N	Exposure assessment	Outcome	Results
Donauer *et al.* 2016 [23]	HOME Study mother-child cohort (Cincinnati, Ohio, 2003-2006)	n=327	Maternal urinary DAP levels (16 and 26 WG).	Bayley Scales of Infant Development at ages 1, 2, 3 years, the Clinical Evaluation of Language Fundamentals-Preschool at age 4, and the Wechsler Preschool and Primary Scale of Intelligence at age 5.	No association between prenatal exposure to OP and cognition at 1-5 years. Points out a possible protection of socioeconomic status and healthier diet.
Millenson *et al.* 2017 [24]	HOME Study mother-child cohort (Cincinnati, Ohio, 2003-2006)	n=224	Maternal urinary DAP levels (16 and 26 WG).	Social Responsiveness Scale (8 years of age).	No association with social behavior, no modification with PON1 status.
Wang *et al.* 2017 [25]	Shandong cohort (China, 2011-2013)	n=237	Maternal (at birth) and childhood urinary levels of DAP.	Developmental quotient scores at 12 and 24 months.	No association at 12 months. Prenatal DAP and DE levels associated with decreased social domain scores and in boys only developmental quotients. Positive associations with postnatal exposure.
Zhang *et al.* 2014 [26]	Shenyang cohort (China, 2011-2012)	n=249	Maternal urinary levels of DAP at birth.	Neonatal Behavioral Neurological Assessment (NBNA).	Inverse association between OP exposure and scores.
Silver *et al.* 2017 [27]	Fuyang cohort (China, 2008-2011)	n=237	Cord blood levels of OP pesticides.	Peabody Developmental Motor Scales between 6 weeks and 9 months of age : gross and fine motor abilites.	Deficits in motor function with chlorpyrifos and naled (used against Zika mosquitoes). Stronger effects in girls.
Silver *et al.* 2018 [28]	Fuyang cohort (China, 2008-2011)	n=237	Cord blood levels of OP pesticides.	Grating visual acuity (VA) and auditory brainstem response (ABR) at 6 weeks, 9 months, and 18 months.	Deficits in vision and head circumference with increasing chlorpyrifos levels but clinical significance unclear.
Kongtip *et al.* 2017 [29]	Thailand birth cohort (Thailand, 2011)	n=50	Maternal urinary DAP levels (at 28 WG).	Bayley Scales of Infant and Toddler Development-III.	DAP levels associated with reduced cognitive composite scores.

Continued on next page

Paper	Study	N	Exposure assessment	Outcome	Results
Cartier *et al.* 2016 [30]	PELAGIE mother-child cohort (Brittany, France - 2002-2006)	n=231	Maternal (<19 WG) and childhood urinary levels of DAP.	WISC-IV at 6 yo.	No association between prenatal exposure and cognitive function, but postnatal DE levels were associated with lower working memory scores.
Cartier *et al.* 2018 [31]	PELAGIE mother-child cohort (Brittany, France - 2002-2006)	n=180	Maternal (<19 WG) and childhood urinary levels of DAP.	FACT at 6 yo.	Prenatal DE levels were associated with decreased contrast sensitivity (in boys only), non-monotonous relation and better scores in girls for DM levels.
Jusko *et al.* 2019 [32]	Generation R mother-child cohort (Rotterdam, The Netherlands, 2002-2006)	n=708	Maternal urinary levels of DAP at each trimester of pregnancy.	Mosaics and Categories sub-tests from the Snijders-Oomen Nonverbal Intelligence Test–Revised at 6 yo.	10 fold difference in DAP concentrations in late pregnancy (>25 weeks) associated with -3.9 IQ. No heterogeneity with PON1 allele status. Suggests a temporal effect and a protective factor status of the consumption of fruits and vegetables.

OBJECTIVES

As presented in the preceding chapters, the human brain is known to be particularly vulnerable during its development and alterations may have consequences during childhood lasting for the entire life.
Therefore, there is a major interest to study and identify risk factors of poorer neurodevelopment, including the environment of the unborn child.

We focused on two environmental chemicals, OP and GE. Both are ubiquitous in the environment of the general population and exposure of pregnant women seem to be widespread and repeated. OP are basically neurotoxicant and GE are poorly investigated for neurotoxicant effects. They both have been associated in epidemiological literature with attention, learning ability or behavioral alterations. This could suggest an alteration of executive functions, in particular inhibitory control, but neural underpinnings of those observed effects are still unknown.

This work has two main objectives: the first one is to see if similar effects to the literature can be observed on motor inhibition of 10-12 years old, by following children from the PELAGIE mother-child cohort, a prospective population based-cohort. The second aim is to use neuroimaging techniques, here fMRI, to investigate neural basis of subtle alterations in the brain, suggested by the existing body of work.

Three studies were conducted to address these objectives:

1. BOLD responses during a motor inhibition task in 10-12 year-old children and associations with attention and inhibition scores of neuropsychological testing.
2. Effects of prenatal exposure to GE on motor inhibition function evaluated by fMRI.
3. Effects of prenatal exposure to OP on motor inhibitory control evaluated by fMRI.

PART II

METHODOLOGIES

1.1. Study protocols

The PELAGIE mother-child recruited pregnant women between April 2002 and February 2006 from the general population by obstetricians, gynecologists, midwives and ultra-sonographers in three districts of Brittany (Ille-et-Vilaine, Finistère and Côtes d'Armor) (PI: S.Cordier). The participation of health professionals was based on voluntary, around 20% of them agreed to participate.
Pregnant women were invited to join the PELAGIE study at a medical check-up during the first trimester of pregnancy. Health professionals gave the inclusion questionnaire to women agreeing to participate (n = 3,592 women; participation rate around 80%). Women filled in the questionnaire at home and sent it back by mail together with a urine sample (first morning void). Women returning the questionnaire after 19 WG have been excluded, given n = 3,421 pregnant women included in the cohort.
They have been followed-up until delivery, n = 21 dropped-out and n = 3,323 gave birth to a live-born singleton. Information about the pregnancy, newborn health were recorded by midwives and pediatricians. Cord blood, placenta and maternal hair samples were also collected before maternity leave (cf. Figure 1.1).
Then, follow-ups took place at age 2 and 6 years. Questionnaires allowed to record information about lifestyle, development and health of the child. Follow-up at puberty age is ongoing.

1.2. The PERINE study

A specific neuropsychological follow-up was performed at ages 6 (PEPSY study) and 10-12 (PERINE study) on a randomly selected sub-cohort (cf. Figure 1.1).
Sub-cohort members were excluded if: 1. the child's gestational age at birth was < 35 weeks of gestation, 2. the child underwent neonatal hospitalization or resuscitation, 3. the child had hypoglycemia at birth, 4. the child had a 5-minute Apgar score <7 at delivery, 5. the child was diagnosed with genetic anomalies, 6. the mother or child died after birth. At 6 years, the participation rate was 65% (n = 243/373 invited families) (PI: C.Chevrier).
When children were aged between 10 and 12 years, they were invited for a new follow-up (PI: F.Pelé). In order to restrict potential risk factors of poorer neurodevelopment, children were excluded if: 1. the mother had smoked or drunk alcohol during the pregnancy, 2. the child suffered from a medical condition that could affect the neurodevelopment (meningitis, meningoencephalis, head trauma with hospitalization), 3. the child was treated by methylphenidate, psychotropic or anti-epileptic treatment, 4. the child presented contraindication to MRI (claustrophobia, braces or metal devices).
Calculating a number of subjects based on *a priori* statistical power was nearly im-

possible at the beginning of the study because of the absence of existing studies to evaluate *a priori* the association estimators. The few existing epidemiological studies at that time included up to 40 children, the targeted number of inclusion for the present study was 100 children.
Expecting a participation rate of 30%, n = 71 children who did not participate to the PEPSY study but were followed-up at ages 2 and 6 years were selected from the PELAGIE cohort with the same criteria as described above.
In total, n = 251 children were invited to participate to the PERINE study, n = 124 (46.4%) refused to participate or were lost to follow-up, n = 26 (9.8%) were excluded due to technical (braces) or medical reasons (meningitis, head trauma) and n = 107 (43.8%) agreed to participate. Among them, n = 101 actually attended the MRI examination.

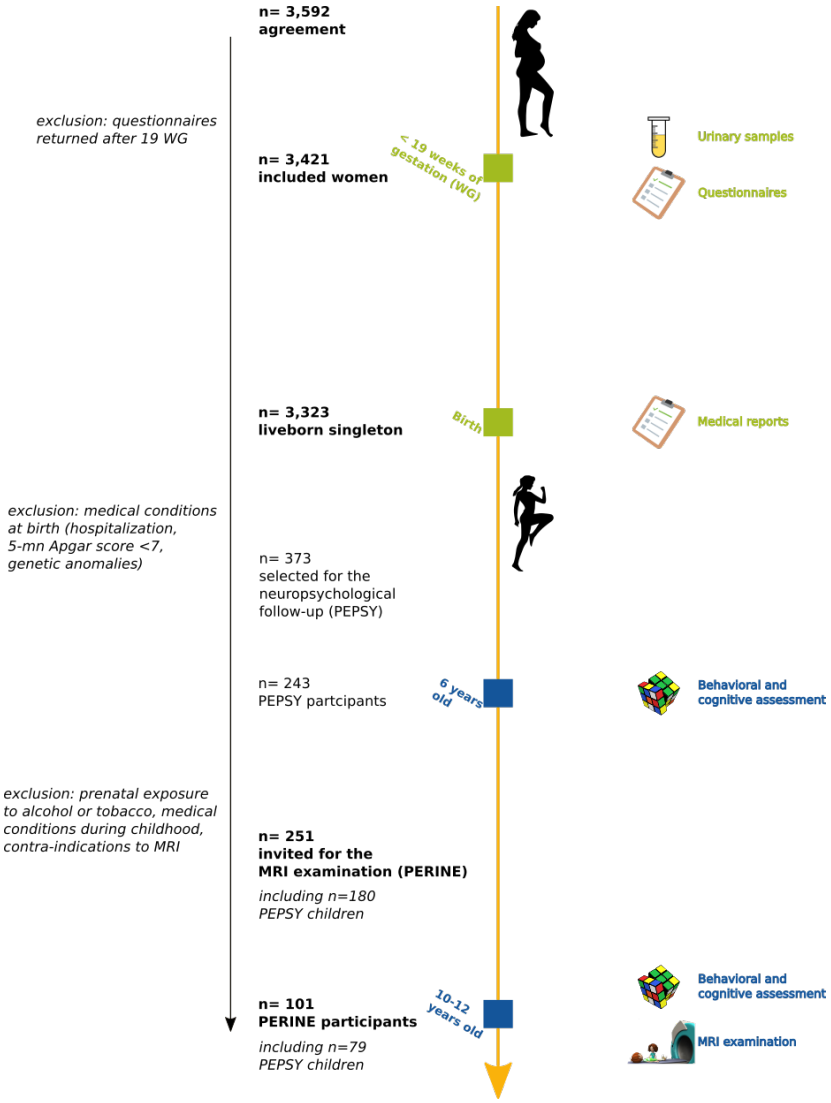


Figure 1.1 – Flowchart of the PELAGIE cohort.

Follow-up took place at the Clinical Investigation Unit of the Rennes University Hospital from November 2014 to November 2016. Proceedings were performed on

Wednesdays when out of the French school holidays periods or during weekdays during holidays periods.
They were standardized as much as possible: all MRI sequences were carried out at the beginning of the testing for almost two hours (45 min-training and 1 hour in the MR scanner). Meanwhile, parents filled out a questionnaire about their child's environment and health.
MRI sequences were followed by cognitive and behavioral assessment with subtests of the WISC and NEPSY batteries and the FACT for visual acuity to contrasts, administered by trained neuropsychologists.

2.1. Use of biomarkers of exposure

Biomarkers can be defined as "any substance, structure or process that can be measured in the human body or its products and may influence or predict the incidence or outcome of disease"[1]. From this definition of Vineis, three types can be determined: biomarkers of exposure (i.e. characteristics of an organism's exposure), effect (i.e. quantifiable changes after exposure in an organism indicating health effect) or susceptibility (i.e. characteristics making an organism more susceptible to an effect). Here, we will only consider the exposure. Unless otherwise specified, the word "biomarkers" will refer to biomarkers of exposure.
Biomarkers can be measured in different matrices: tissues, fluids (urine, blood, breast milk) or skin appendages (hair, nails); as metabolites or parent compounds.
The main advantage of exposure assessment with biomarkers is to aggregate all sources of exposure to estimate the substance impregnation.

The two compounds of interest, GE and OP have been measured in maternal urines as their respective metabolites. Since they both have a short half-life (up to a couple of days), the concentration reflects the quantity of chemicals in the maternal body at that time. First morning void, were collected before 19 WG in a 10-mL test tube (95×16-mm polypropylene, with wing plug), stabilized with chlorhydric or nitric acid to prevent bacterial multiplication and frozen at -20°C until chemical analyses.
Urine samples were not available for six women because they had already been used for other urinary assays.

2.2. Exposure to glycol ethers

GE were assessed by gas chromatography coupled to mass-spectrometry (GC-MS) (HP 7890A; HP 7000C; Agilent) at the LABOCEA (Laboratoire public Conseil, Expertise et Analyse en Bretagne). This method allows the simultaneous detection and measure of 6 alkoxycarboxylic acids of the most used GE in France at the time of the study: MAA, EAA, EEAA, BAA, PhAA and 2-MPA. Concentration range linearity was observed from 0.01 to 1.00 mg/L for the urinary GE metabolites. The average recovery was 99–104%, and the coefficients of variation based on duplicates ranged from 15% to 20%. The limit of detection (LOD) was 0.003 mg/L for all metabolites.
Analyses were performed in 2013 (n = 54) and 2017 (n = 19), we found no mean differences between the two series of measurements.
Some samples (n = 22) were analyzed in 2006 by the laboratory of toxicology and genopathy (CHRU Lille) with the same GC-MS method but a limit of detection of 0.05 mg/L.

1. Vineis, "Sources of variation in biomarkers".

2.3. Exposure to organophosphate insecticides

Six nonspecific DAP metabolites of OP insecticides (DEP, DETP, DEDTP, DMP, DMTP and DMDTP) were analyzed with liquid chromatography-electrospray ionization tandem mass spectrometry (LC-SMS) after solid-phase extraction (SPE) at the LABOCEA.

Analyses were performed in 2007-2008 (n = 54), 2013 (n = 22) and 2017 (n = 19). The limits of quantification (LOQ) for the chemical analyses performed in 2007-2008 (hereafter referred as series n°1) were 1.25, 1.7, 0.02, 0.2, 1, and 0.45 μg/L for, respectively, DEP, DETP, DEDTP, DMP, DMTP, and DMDTP. For series n°2 and n°3, respectively analyzed in 2013 and 2017, values between the LOD and the LOQ were available. The LODs were 0.2, 0.1, 0.005, 0.06, 0.32, and 0.13 μg/L for, respectively, DEP, DETP, DEDTP, DMP, DMTP, and DMDTP.

Metabolite concentrations were converted from μg per liter to their molar concentrations (nanomoles per liter, nM). Concentrations were summed to obtain overall concentrations of diethylphosphate metabolites (DE; sum of DEP, DETP, and DEDTP), dimethylphosphate metabolites (DM; sum of DMP, DMTP, and DMDTP), and all six nonspecific DAP metabolites.

3.1. Paradigm of the Go/No-Go task

Motor inhibition was evaluated with a visual Go/No-Go task. This event-related task, adapted from a study of Mostofsky *et al.*[1], minimizes cognitive demands other than motor execution and response inhibition and can be easily performed in children[2].
Children completed the task in a Magnetic Resonance (MR) scanner. First, short practices were performed in front of a computer then in a mock MR scanner to provide instructions and accustom the child to the scanner environment.
The task consisted in the successive presentation of green and red smileys on a screen. Children were asked to press a button as quickly as possible when seeing a green smiley but had to refrain when seeing red smileys. The ratio of no-go cues (red smileys, n = 54) over go cues (green smileys, n = 246) elicited a dominant response. The task was split into two runs of 5 minutes each. Each run consisted of 150 trials and four 10-second rest periods. A trial was the consecutive presentation of a smiley (duration of 200 ms) and a cross fixation point (duration of 1300 ms). A response was allowed until the next trial began, giving a constant inter-stimulus interval of 1500 ms. Smileys appeared in a pseudo-random order: trials at the beginning of runs or following resting periods had to be go cues. Go cues had to occur at least three times in a row and no-go cues had to occur individually or at most twice in a row (cf. Figure 3.1).
The task was implemented and recorded using E-Prime v.2.0.8 Professional (Psychology Software Tools, Pittsburgh, PA, USA) and presented using the Nordic Neurolab Solution (Nordic Neurolab, Bergen, Norway).

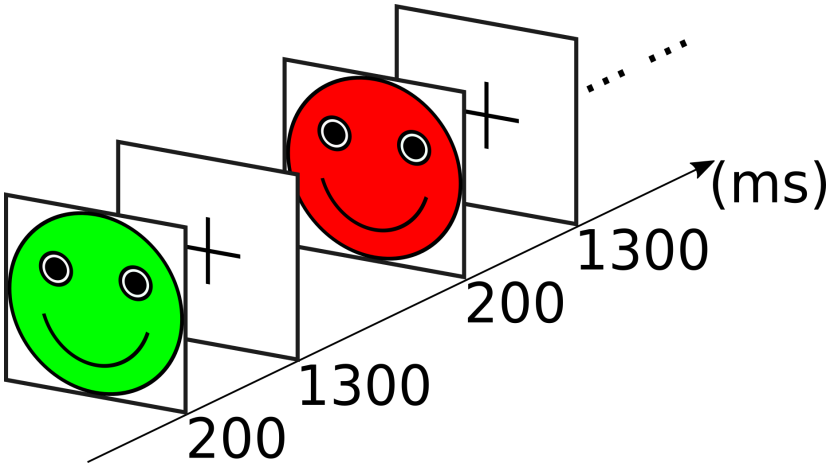


Figure 3.1 – Paradigm of the Go/No-Go task.

1. Mostofsky et al., "fMRI evidence that the neural basis of response inhibition is task-dependent".
2. Réveillon et al., "Functional neuroimaging study of performances on a Go/No-go task in 6- to 7-year-old preterm children"; Suskauer et al., "Functional Magnetic Resonance Imaging Evidence for Abnormalities in Response Selection in Attention Deficit Hyperactivity Disorder".

3.2. Neuropsychological assessment: performance indicators

To test whether or not motor inhibition may differ according to levels of exposure to environmental contaminants, we built indicators of performance at the task. The Go/No-Go task does not come with validated scores and we aimed at investigating different aspects of the performance.

First, we used commission rate (incorrect answers at no-go cues) to evaluate the ability of the child to inhibit its gesture. We also measured the response latency to answer at go cues to investigate a possible impulsivity of children, or at contrary, compensatory strategy (i.e. children slowing down their answers to inhibit more easily). We found an inverse correlation between commission rate and response latency (i.e. the lower the commission rate, the higher the response latency). Thus, we built a composite performance score by subtracting the average response latency from the accuracy of motor inhibition, following standardization. The accuracy of motor inhibition was assessed by the sensitivity index, d', which subtracts the standardized (z score) commission rate from the standardized hit rate (correct answers for go cues), as proposed by Collignon *et al.* (cf. Equation 3.1)[3].

$$\begin{aligned} \mathrm{d}' &= \mathrm{Z}(\mathrm{hit\ rate}) - \mathrm{Z}(\mathrm{commission\ rate}) \\ \mathrm{composite\ performance\ score} &= \mathrm{Z}(\mathrm{d}') - \mathrm{Z}(\mathrm{averaged\ response\ latency}) \\ &\qquad (\mathrm{Z} : \mathrm{standardized\ value}) \end{aligned} \tag{3.1}$$

Children with the highest composite performance scores were considered to efficiently perform the task (fast with a high hit rate, with few commissions), whereas children with the lowest scores were slow and found it difficult to inhibit their response to no-go cues.

3.3. Brain activities related to motor inhibition: contrast(s) of interest

Acquisition

Neural activity related to motor inhibition was evaluated using BOLD fMRI. Scanning was completed on a 3 T MR Scanner (Magnetom Verio, VB17, Siemens, Erlangen, Germany) using a 32-channel receiver head coil. Functional images were acquired using gradient echo-planar imaging with repetition time (TR) of 2500 ms and echo time (TE) of 30 ms, a 90° flip angle, and 110 volumes per run. Each volume was composed of 34 axially oriented 4 mm-interleaved slices, covering the whole brain. Scans with a voxel size of 2×2×4 mm^3 were based on a 110×110 acquisition matrix (220×220 mm^2 field of view). Two children who moved repeated the sequence but learning effects are not expected for this task and we did not observe any improve-

3. Collignon et al., "Women process multisensory emotion expressions more efficiently than men".

ment in performance during the two sessions.
High-resolution 3D anatomical images were obtained using T1-weighted MPRAGE at 1 mm^3 resolution for anatomical referencing (TR of 1900 ms, inversion time of 900 ms, TE of 2.26 ms, 9° flip angle).

Image pre-processing

Image preprocessing was performed using Matlab v.9.2 (Mathworks. Natick. MA. USA) and SPM12. Anatomical template was built using SPM8[4].
Anatomical images were segmented into cerebrospinal fluid, GM and WM, bone, soft tissues and air by using tissue probability maps. Head motion artifacts on anatomical images and segmented tissues were checked visually for each subject.
Functional images were first corrected for differences in acquisition time between slices and realigned with a six-parameter (rigid body) approach. A mean volume was created and co-registered to the subject high-resolution anatomical image.
A common group template was created from individual anatomical images using DARTEL to obtain a common space specific to our population and age range, and spatially normalized to the Montreal Neurological Institute (MNI)-labeled space for comparison to the literature[5]. Functional images were normalized to the created anatomical template and smoothed using a 8-mm^3 Gaussian kernel. Head movements during the functional scan were evaluated by six parameters calculated during the realignment step. Two children had head motion superior to 2 mm (in-plane size of an acquisition voxel) and their images were excluded for further analyses (cf. Figure 3.2).

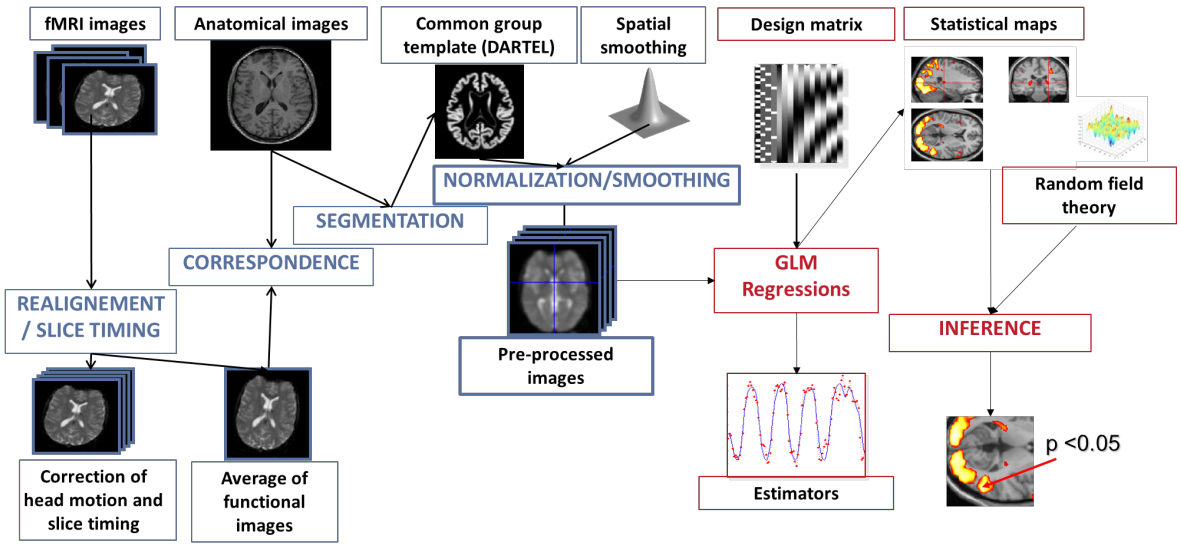


Figure 3.2 – Image analysis using SPM[4].

4. Friston, *Statistical parametric mapping*; Wellcome Centre for Human Neuroimaging, *Statistical Parametric Mapping (SPM)*
5. Ashburner, “A fast diffeomorphic image registration algorithm”.

Modeling brain activation

The HR was modeled from trials (i.e. go cue and no-go cue), implicit baseline (i.e. resting periods and the time between trials), and motion regressors using the Statistic Parametric Mapping (SPM) canonical HR function and its temporal derivative. This HR function is the subtraction of two gamma functions. The first γ function models the peak of intensity with a latency of 6 seconds and the second the undershoot during the recovery period with a latency of 16 seconds. The use of a temporal derivative allows for variations in peak latency, while providing comprehensive models for the response.
Scanner drift was modeled with a discrete cosine transform set (128-second cut off). Temporal autocorrelation was accounted for using an autoregressive AR(1) model over the whole brain.
Even at rest, the brain remains active so we used two contrasts to be as specific as possible of our brain function of interest. First, we modeled go and no-go events, independently of the subject's response. Secondly, we modeled successful no-go, failed no-go and go conditions. Failed go conditions were around 1% and were therefore considered negligible.
For descriptive purposes, we used "Successful Go", "Successful No-Go" and "Failed No-Go". We extracted "No-Go vs Go" activations, representing activation amplitudes that were higher for inhibition than motor tasks, when children perceived the inhibition demand. Secondly, we built contrast images for "Successful No-Go vs successful Go", when children were able to inhibit and stop their answers.

4.1. Homogenizing sampling conditions of exposure assessment

Due to the cost of the chemical analyses, urinary samples were analyzed for successive research studies, in 2006-2008, 2013 and 2017. Thus, there were different sampling conditions, in particular the variability of storage duration (even if degradation of frozen samples is not expected) and an improvement of the analytic methods. Therefore, we could assume an heterogeneity of the concentrations, depending on the sampling conditions, yet not the true level of exposure (i.e. not differential errors). Moreover, exposure concentrations are particular quantitative data with values below the LOD (also called "undetected values") due to the limits of analytic methods for measuring accurately low amounts of chemicals. It results in informative left censoring data, which need to be taken into account to not bias association estimators.

For GE metabolites, the latest series (series 2 in 2013 and series 3 in 2017) were similar (i.e. same LOD at 0.003 mg/L and the means of distributions did not statistically differ) but series 1 presented poor detection rates (due to an higher LOD at 0.05 mg/L). At the exception of PhAA which was detected in 95% samples (vs 98% for series 2 and 3), other GEs had detection rates between 0 and 27% (vs 90–98%). It was nearly impossible to impute values from series 1. Samples from the series 1 (n = 22) were excluded from further association analyses with GE in order to limit the heterogeneity of the measurements and preserve statistical power.
There was less heterogeneity in chemical assay of OP metabolites. In order to maximize the sample size and the statistical power, we chose to statistically standardize sampling conditions. Based on Mortamais *et al.*[1], the purpose of this method is to obtain levels of metabolites that "would have been observed if samples [here, maternal urine] were collected and analyzed in the same conditions". First, we estimated the effects of the sampling day, duration of storage before and after freezing, creatinine levels (representing dilution effect) using Tobit regressions, to deal with left censored data with different LOD. Regressions were adjusted on season and year of inclusion, which may be associated with the true exposure (i.e. temporal variability in the use of insecticides). Concentrations were corrected as if maternal urine had been collected during weekday, with similar dilution (median of creatinine distribution), stored less than 24 hours before freezing and analyzed less than 5 years after collection. As shown on Figure 4.1, this method has limited the effects of sampling conditions by reducing heterogeneity between measurement series.

Then, one way to take into account left censored exposure data is to impute the values below the LOD. GE and OP were log-normally distributed, which is commonly found with environmental pollutants. We first estimated the parameters of the distri-

1. Mortamais et al., “Correcting for the influence of sampling conditions on biomarkers of exposure to phenols and phthalates”.

bution (mean and standard deviation) from the uncensored (i.e. quantified) values by maximum likelihood estimation (MLE). We generated values from this distribution and randomly imputed missing values.

We assessed the linearity hypothesis of the relation between exposure and inhibitory control with restricted cubic splines. When the relation was not linear, exposures were categorized following the tertile of distribution or in <LOD; <median detected value; $\geqslant$ median detected value if there were more than 30% of undetected values).

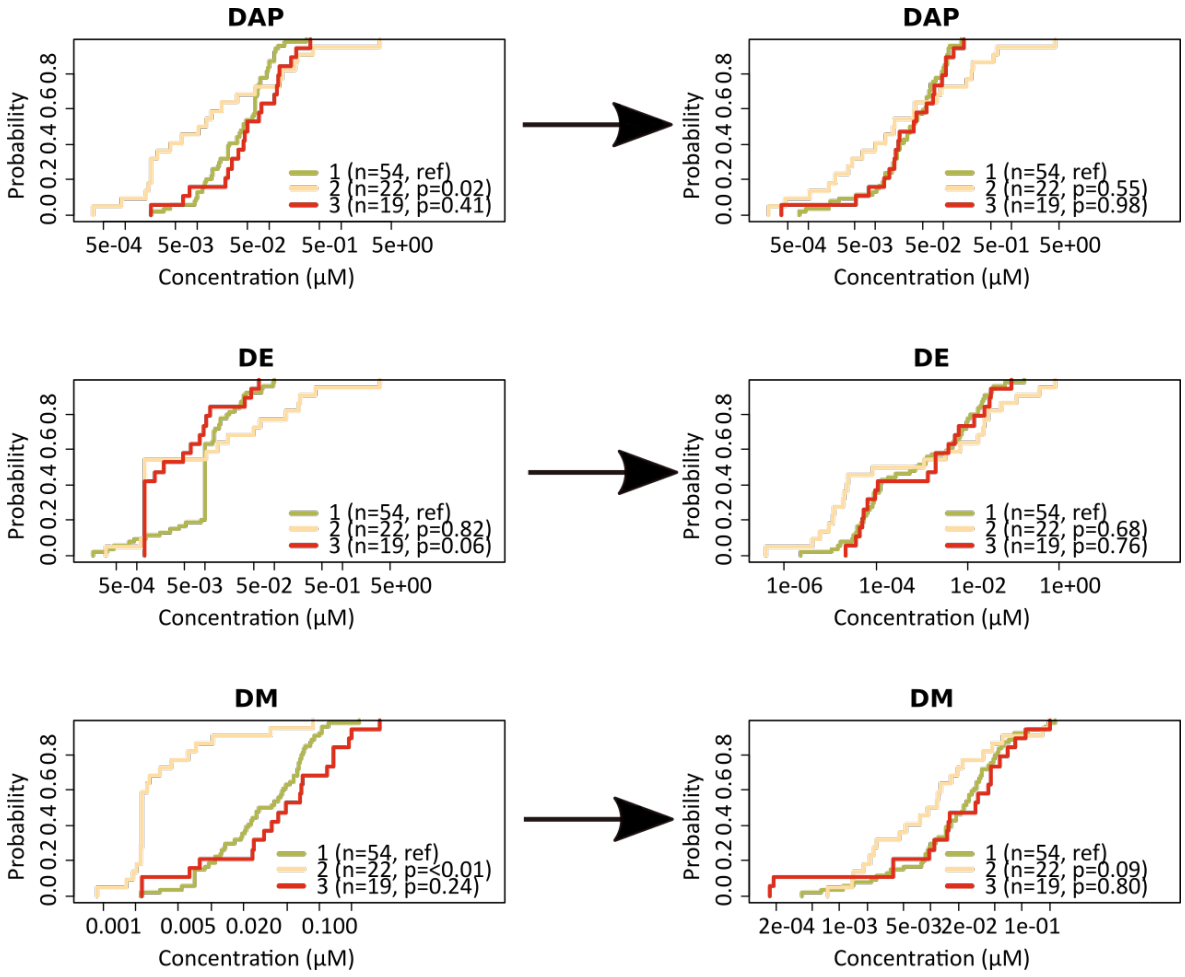


Figure 4.1 – Probability vs. concentration of OP metabolites by series before (left part) and after standardization (right part).

4.2. Taking into account confounding factors

Self-reported questionnaire allowed collecting various covariates about the mother (maternal age, educational level, parity, pre-pregnancy BMI, tobacco and alcohol consumption at the beginning of pregnancy. Most of child characteristics were recorded at birth (sex, gestational age at birth) and at ages 2, 6 and 10-12 by questionnaires.

Regarding the relatively small size, our strategy was to preserve the statistical power

by limiting the number of adjusting factors.
Some well-known risk factors of poorer neurodevelopment were taken into account by restriction in excluding children with prenatal exposure to tobacco and alcohol, medical conditions or medical treatment. Other potential confounders were included in regression models as adjusting factors chosen *a priori* from the literature or by statistical criteria (further details of adjustment are presented in the articles). There were very few missing data on covariates which were imputed by the mode of the distribution.

4.3. Statistical modeling

We used linear regressions to analyze the association with concentrations of exposure and performance indicators (i.e. reaction time, commission rate, and composite performance score).
The HR during the motor inhibition task was modeled by mixed effect generalized linear regressions to investigate the interaction between exposure and the effects of the motor inhibition task on cerebral activity. Statistical significance was assessed for cluster-wise significance, defined by random field theory (family-wise error rate (FWER) corrected p=0.05) to account for 3D spatial autocorrelation, using an uncorrected one-sided cluster-defining threshold of p=0.01[2]. Briefly, from a random 3D spatial distribution of values (cf. Figure 4.2) and given the number of voxels, the roughness of the data and our threshold (here α risk at 5%), we were able to determine statistically significant clusters, limiting the risk of false positives.

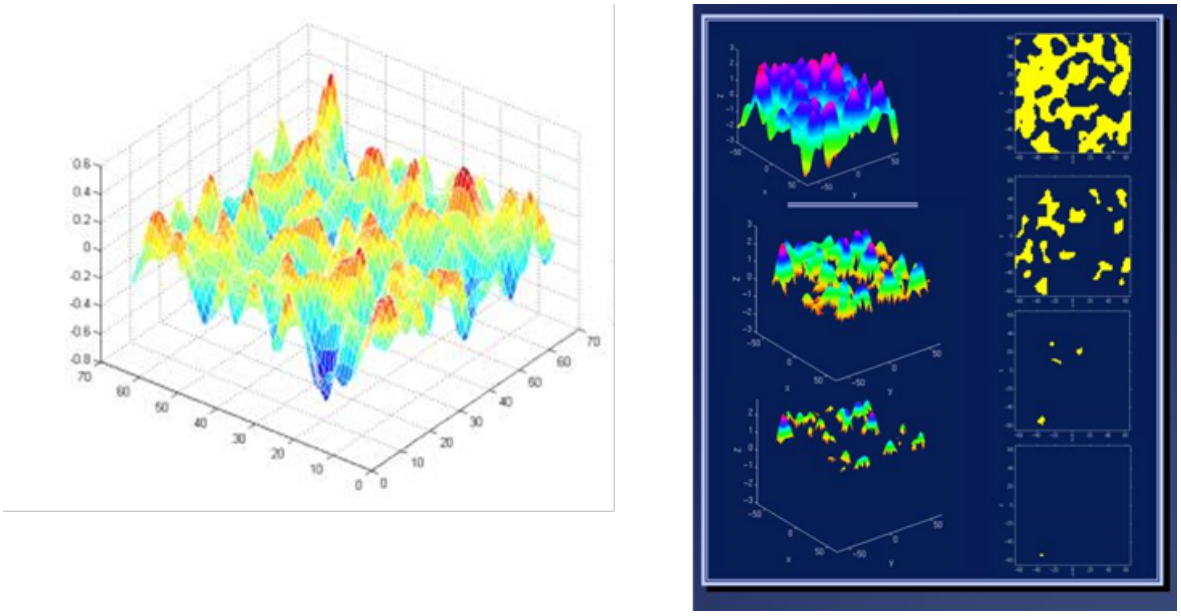


Figure 4.2 – Continuous random field[4].

We tested sex-specific effect for the association between OP and neurodevelop-

2. Nichols and Hayasaka, “Controlling the familywise error rate in functional neuroimaging”.

ment because there was some literature about this hypothesis, whereas it was not the case for exposure to GE.

We investigated several molecules in our studies, multiplying statistical testing and increasing the risk α (usually 5%), which determine the probability to falsely conclude to an association. There are several methods to correct the risk of false positives (e.g. Bonferroni, false discovery rate (FDR) or FWER) but they also limit the statistical power by increasing the risk β that determine the probability to falsely reject an association. Our studies being exploratory, we chose to preserve statistical power and to not correct for multiple testing.

PART III

RESULTS

MOTOR INHIBITION BOLD RESPONSES AND NEPSY SCORES

Status: in preparation

This article is beyond the scope of epidemiology and still on-going. Only the "Material and methods" and "Results" sections are presented here.

Binter AC, Simon G, Chevrier C, Pelé F, Bannier E. BOLD responses during a motor inhibition task and NEPSY scores at the age of 10 to 12 years (PELAGIE mother-child cohort).

1.1. Résumé

Objectifs: L'objectif de ce travail est de décrire les activations cérébrales pendant une tâche de Go/No-Go d'enfants de 10 à 12 ans recrutés en population générale et d'évaluer les corrélations entre ces activations et les scores de tests neuropsychologiques d'attention et d'inhibition.

Méthodes : Une tâche visuelle de Go/No-Go a été présentée aux enfants de la cohorte PELAGIE et cette étude est restreinte aux droitiers (n = 87). Les scores d'attention sont issus des sous-tests d'attention visuelle et attention auditive de la NEPSY. Les scores d'inhibition sont construits à partir des performances à la tâche de Go/No-Go. La tâche de Go/No-Go a été réalisée pendant une séquence d'IRM fonctionnelle. Cinq contrastes ont été construits à partir des images : "Go réussis", "No-Go réussis", "No-Go échoués", "No-Go réussis vs Go réussis" et "No-Go vs Go réussis". Les estimateurs extraits du modèle général linéaire ont permis d'obtenir les clusters activés pour chaque contraste. Les activations pour chaque région d'intérêt ont été moyennés. Les corrélations entre les activations et les scores d'attention et d'inhibition ont été estimées par des coefficients de Spearman entre les estimateurs des régions d'intérêt et les scores.

Résultats : A partir des contrastes spécifiques à l'inhibition ("No-Go réussis vs Go réussis" et "No-Go vs Go réussis"), nous avons observé des activations cérébrales dans les régions frontales (bilatéralement) dont le cortex inférieur frontal latéral, le cortex cingulaire antérieur et l'aire motrice supplémentaire. Les scores neuropsychologiques étaient faiblement corrélés aux activations cérébrales.

1.2. Motor inhibition BOLD responses and NEPSY scores

The article resulting from the study is inserted on the next pages.

Aim: To describe answers at a Go/No-Go task of 10-12 years old children during brain functional imaging, recruited in the general population and assess correlation between brain activations and neuropsychological tests.

Material and methods

Study population

Children were recruited from a prospective mother-child cohort in Brittany (France). The PELAGIE cohort included 3421 women at the beginning of pregnancy in general population between 2002 and 2006. The children were followed during their development and environmental as well as health condition data were collected. Children had to be born at term (delivery after 35 weeks of amenorrhea) and to present no major condition at birth (neonatal hospitalization, hypoglycemia, 5 minute-Apgar score <7), no prenatal exposure to tobacco, alcohol or medical treatment during childhood which could affect their neurodevelopment (methylphenidate, psychotropic or antiepileptic drugs, etc.) (Cartier et al. 2016; Garlantézec et al. 2009). A sub-cohort of 87 children aged between 10 and 12, all right-handed were included in the present study.

Parents and child participants provided written informed consent and the appropriate ethics committees approved the study (Committee for the protection of persons- n°2013-A01420-45, French Consulting Committee for the Treatment of Information in Medical Research, n°09.485, and the French National Commission for the Confidentiality of Computerized Data, n°909347).

Motor inhibition task

All children completed a 10-minute task in the MR scanner. First, a short practice in front of a computer then in a mock MR scanner was performed to display the instructions and accustom the child to scanner environment.

The event-related visual Go/No-Go task was adapted from Mostofsky et al. (Mostofsky et al. 2003). Green and red smileys were successively presented on a screen. Children were asked to press a button with their right thumb as quickly as possible when seeing a green smiley, but had to refrain otherwise. The task was split in two runs of 5 minutes each. Each run consisted of 150 trials and four 10-second resting periods. A trial was the consecutive presentation of a smiley (duration of 200 ms) and a fixation cross (duration of 1300 ms). A response was allowed until the next trial began, giving a constant inter-stimulus interval of 1500 ms. Smileys appeared in a pseudo-random order: trials at the beginning of runs or following resting periods had to be Go cues. At least at three Go cues had to occur in a row and No-Go cues had to occur individually or by two. The ratio of No-go cues (red smileys) over Go cues (green smileys) was 1:4, in order to elicit a dominant response. The task was implemented using E-

Prime v.2.0 (Psychology Software Tools, Pittsburgh, PA, USA) and presented with the Nordic Neurolab Solution (Nordic Neurolab, Bergen, Norway).

Neuropsychological scores

Children performances at the Go/No-Go task performed in the MR scanner were evaluated by omission rate (no answers to go cues) and commission rate (incorrect answers to no-go cues) and response latency (average reaction time for the correct answer of go cues). Responses below 200 ms were considered as anticipatory responses and excluded from performance calculation.

All children also completed a battery of neurodevelopmental tests outside the MR scanner with trained neuropsychologists (Korkman et al. 1998). In particular, auditory and visual attention subsets of the NEPSY were used in the present analysis for their relevance in the context of motor inhibition evaluation. Visual attention subset assesses speed and accuracy to locate a specific item in a group of elements: children were asked to circle specific images on a page of pictures that included distracters, with scores scaling from 0 to 45. Auditory attention subset consisted of two parts: “auditory attention”, scaling from -180 to 60, measuring selective attention: children had to place a red square into a box when hearing “red” and “response set”, scaling from -180 to 72, assessing flexibility and shifting pattern abilities: children had to place a red square into a box when hearing “yellow” and vice versa. A new instruction (place a blue square into a box when hearing “blue”) served as distractor. Added both together, auditory attention score scales from -360 to 132. Higher scores indicate a better attention.

Imaging procedure

Scanning was completed on a 3 T MR Scanner (Magnetom Verio, VB17, Siemens, Erlangen, Germany) using a 32-channel receiver head coil. High-resolution 3D anatomical images were obtained using T1-weighted MPRAGE at 1 mm^3 resolution for anatomic referencing (repetition/inversion/echo times: 1900/900/2.26 ms, 9° flip angle). Functional images were acquired using gradient echo-planar imaging (EPI) with repetition/echo times of 2500/30 ms, a 90° flip angle, parallel acquisition (generalized autocalibrating partially parallel acquisition (GRAPPA) reconstruction with an accerelation factor of 2, and 110 volumes per run. Each volume was composed of thirty-four 4 mm-interleaved slices oriented along the AC-PC line, covering the whole brain. Scans with a voxel size of 2*2*4 mm^3 were based on a 110*110 acquisition matrix (220x220 mm^2 field of view (FOV)). Operators assessed image quality during the acquisition, two children who moved repeated the sequence. Learning effects are not expected for this task and we did not report performance improvement during the two sessions. The scan duration was 30 minutes.

Image preprocessing

The functional and structural data were preprocessed and analyzed using SPM12 (Wellcome Department of Imaging Neuroscience, London, England) in MATLAB (Mathworks, Inc., Sherborn, MA). Anatomical images were segmented into cerebrospinal fluid, grey matter and white matter, bone, soft tissues and air by using tissue probability maps. Head motion artifacts on anatomical images and quality of segmentation were checked visually for each subject.

The fMRI volumes were corrected for the acquisition time delay between slices (slice timing). Rigid realignment was then performed to register the volumes to the mean fMRI volume to compensate for subject motion and ensure voxel-to-voxel correspondence across time. The mean fMRI volume was co-registered to the participant's structural image, using a rigid body transformation model (6 parameters). Head movements were evaluated by six parameters calculated during the realignment step. Only participants with a head motion less than 2mm (in-plane size of an acquisition voxel) were included in this analysis (n=83).

A customized template was created based on the individual gray matter segmentations using a diffeomorphic method known as DARTEL (Ashburner 2007). DARTEL uses an iterative process to progressively refine warping parameters describing the transformation from individual subject space to a common space. During this procedure flow fields were calculated and subsequently applied to the native gray matter image of each subject. Next, the affine transformation from the customized template space to MNI space was calculated and applied to the individual structural image.

fMRI data analyses

Hemodynamic response was modeled from go cues with correct answers, no-go cues with correct answers and no-go cues with incorrect answers - i.e. commission errors, separately, using a canonical hemodynamic response function (HRF) and its temporal derivative. Omissions (no answer to Go cues) were not explicitly modelled due to low rate of errors. No differences were found between the two runs, neither for the performance score and response latency, nor for contrast estimates at cluster wise FWER corrected – $p<0.05$), allowing concatenating the two runs, with 2 additional regressors to model the runs. Six motion regressors (3 translations, 3 rotations) were also included in the design matrix.

Scanner drift was modeled with discrete cosine transform (DCT) set (128 second- cut off) and temporal autocorrelation was accounted for using an autoregressive AR(1) autocorrelation model over the whole brain.

Maps were extracted for successful Go, successful No-Go and failed No-Go conditions for each subject by voxel-wise multiple regressions estimated by restricted maximum likelihood (ReML) method and used as inclusive masks for contrast images.

“Successful Go”, “Successful No-Go”, “Failed No-Go”, “Successful No-Go vs successful Go” and “No-Go vs successful Go” images were contrasted (Figure I).). We distinguished the “Successful No-Go vs successful Go” and “No-Go vs successful Go” contrast, considering that the first one is specific of the “true”, successful, inhibition and the second one represents the pure instruction, i.e. inhibition condition.

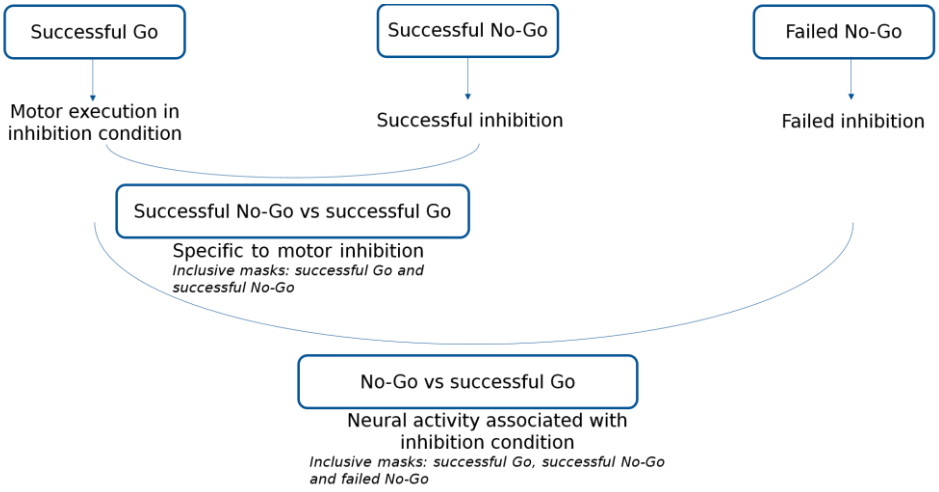


Figure I: Contrasts of interest

Statistical significance was assessed for cluster-wise significance defined by random field theory (FWER corrected p=0.05) using an FWER corrected one-sided cluster-defining threshold of p=0.05.

Correlation between neuropsychological scores and neural activity

We extracted regions of interest (ROI) assess correlation between activation clusters and neuropsychological scores (Table 1). First, we extracted mean beta estimates of clusters activated in successful Go contrast, Successful No-Go vs successful Go and No-Go (failed and successful) vs successful Go contrasts. We correlated values with inhibition (commission rate) and visual attention score, auditory attention and response set (NEPSY) scores respectively. Correlations tests were conducted with Spearman coefficients at alpha risk=0.05.

Table 1: Correlations investigated between neural activity during the Go/No-Go and attention scores at neuropsychological NEPSY test.

Defined ROI	**Scores of interest**
Successful Go contrast	Execution (omission rate and response latency) and attention (NEPSY) scores
Successful No-Go vs successful Go contrast	Inhibition (commission rate) and attention (NEPSY) scores
No-Go vs successful Go contrast	

R software v.3.5.2 (https://www.R-project.org/) and SPM12 (http://www.fil.ion.ucl.ac.uk /spm/) were used for statistical analyses.

Results

Population characteristics

Children included in this follow-up sub-study were mainly girls (n=47), all were between 10.1 and 11.8 years-old with a median age of 10.8 years-old. They performed the Go/No-Go task with a median response latency of 398 ms. The commission rate (median of 25.9%) was much higher than the omission one (median of 1.6%).

Visual attention scores went from 12 to 30 with a median value of 21, whereas auditory attention scores went from 91 to 132 with a median value of 122 (from 42 to 60 and 38 to 72 for auditory and response set parts respectively). We found no difference of performances according to child's sex (Table 2).

Table 2: Characteristics of the study population (n=83).

	All (n=83) Median (min-max)	Boys (n=36) Median (min-max)	Girls (n=47) Median (min-max)	Pvalue
Age (in years)	10.8 (10.1-11.8)	10.8 (10.4-11.5)	10.8 (10.1-11.8)	0.67
Go/No-Go performances				
Omission rate (in %)	1.6 (0-30)	2.4 (0-30)	1.6 (0-11.6)	0.13
Commission rate (in %)	25.9 (3.7-53.7)	25.9 (3.7-51.9)	24.1 (3.7-53.7)	0.56
Response latency (in ms)	398.4 (320.7-954.2)	406.2 (335.3-954.2)	392.6 (320.7-595.4)	0.08
NEPSY: Attention and Executive functions				
Visual attention score	21 (12-30)	20 (13-30)	23 (12-30)	0.21
Auditory attention score	122 (91-132)	118 (96-130)	123 (91-132)	0.26

Auditory attention part	58 (42-60)	58 (43-60)	58 (42-60)	0.99
Response set part	65 (38-72)	62 (53-71)	65 (38-72)	0.12

Cerebral activity during the Go/No-Go task

When children answered at Go cues, BOLD responses were observed mainly in bilateral occipital regions (visual cortex), supplementary motor area (SMA), predominantly in the left hemisphere, left precentral gyrus and right anterior cerebellum (motor regions). We also reported neural activity in right inferior parietal cortex (regions involved in attention) and bilateral medial frontal regions (inhibition) (Table 3, Figure II).

Successful No-Go contrast showed activations in bilateral middle frontal and insular cortices, and SMA (motor inhibition) and right parietal and temporal gyri (regions involved in attention). We also observed cerebral activity in left pre/post-central area and bilateral anterior cerebellum (motor regions) and right occipital area (involved in vision processing) (Table 4, Figure III). With Failed No-Go contrast, we observed activations in bilateral cingular, insular and inferior frontal cortices (inhibition network) and bilateral temporal and inferior parietal regions (attention network). There are also BOLD responses in bilateral anterior parts of cerebellum and occipital regions (respectively motor and visual regions) (Table 5, Figure IV).

For “Successful No-Go versus successful Go” contrast, we showed increased neural activity in left insular, right frontal and bilateral cingular cortices (involved in inhibition) and bilateral occipital, right inferior parietal and temporal gyri (attention network, including visual attention). We also report activations in bilateral anterior part of cerebellum and left thalamus (Table 6, Figure V).

For “No-Go versus successful Go” contrast, there are similar activations in bilateral insular, left frontal and bilateral cingular cortices (inhibition regions) and right occipital, bilateral inferior parietal and temporal gyri, bilateral anterior part of cerebellum and right thalamus (attention regions) (Table 7, Figure VI).

Neural activities and neuropsychological scores

With successful Go contrast we found only a trend toward correlation between activity in right inferior parietal region and response latency (rho=0.20, p=0.08).

Activations in left insular regions associated with “Successful No-Go vs successful Go” contrast were slightly correlated with visual attention score (rho=0.24, p=0.03 and rho=0.23, p=0.04) and commission rate (rho=0.24, p=0.02).

With “No-Go vs successful Go” contrast, we reported correlations between activations in cingular regions and commission rate (rho=-0.31, p<0.01 and rho=-0.28, p=0.01) and attention scores (visual, rho=0.23, p=0.04, auditory attention rho=0.23, p=0.03 and response set rho=0.29, p=0.01). There are similar correlations with activations in inferior frontal/insular regions: commission rate, rho=-0.25, p=0.02 and auditory attention, rho=0.24, p=0.03. BOLD responses in right anterior cerebellum were also correlated with response set score (rho=0.26, p=0.02).

Table 3: Neural activity when answering at Go cues (n=83) (FWER correction at cluster level, p<0.05).

Cluster			**Cluster foci**			
Structure	**Hemisphere**	**Volume (in mm³)**	**X**	**Y**	**Z**	**t-value**
Pre/post-central region	L	5088	-36	-18	56	8.09
Occipital region (inferior/middle/sup)	R	3408	22	-102	8	8.92
	L	3472	-20	-94	0	7.55
Supplementary motor area (SMA)	L	1184	-4	-2	60	6.34
Anterior part of cerebellum	R	1072	14	-50	-20	6.86
	R	352	34	-52	-28	5.17
Medial superior frontal region	R	144	12	32	64	5.36
Supramarginal gyrus	R	144	52	-54	52	5.38

Cluster volume is reported in mm³. Cluster foci of highest t-value at FWER-corrected p<0.05, cluster threshold 5. Coordinates are reported in mm in MNI space.

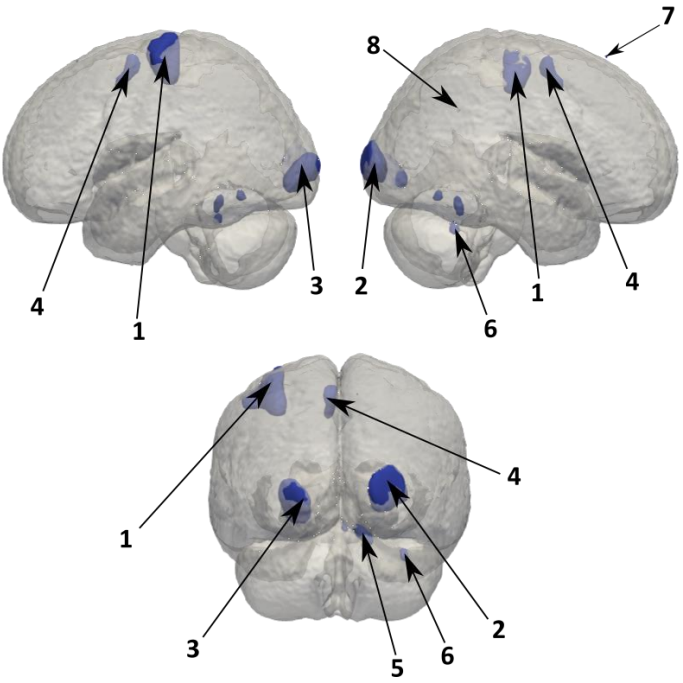


Figure II: Neural activity when answering at Go cues (n=83) (FWER correction at cluster level, p<0.05).

1: Left pre/post-central region, 2: Right occipital region, 3: Left occipital region, 4: Left supplementary motor area, 5 and 6: Left anterior exterior cerebellum

Table 4: Neural activity when inhibiting correctly No-Go stimuli (n=83) (FWER correction at cluster level, p<0.05).

Cluster			**Cluster foci**			
Structure	**Hemisphere**	**Volume (in mm^3)**	**X**	**Y**	**Z**	**t-value**
Anterior part of cerebellum	L	34424	-40	-60	32	9.68
	R	160	14	-60	-44	5.24
Inferior parietal region	R	16928	34	-58	44	8.36
Supplementary motor area	L/R	13248	4	6	52	8.15
	R	12336	42	30	36	7.58
Middle frontal cortex	R	80	32	56	0	5.01
	L	368	-32	30	28	5.39
Occipital region (inferior/middle)	R	6864	18	-102	4	9.15
Putamen/Insula	R	4544	26	14	8	6.77
Thalamus	R	176	8	-6	4	5.12
Putamen	L	3056	-24	6	-4	6.60
Thalamus	L	208	-8	-4	4	5.72
Temporal region (middle, inferior)	R	3808	60	-26	-8	6.78
Pre/post-central region	L	2464	-34	-22	52	7.89
	L	96	-52	-20	52	5.28

Cluster volume is reported in mm^3. Cluster foci of highest t-value at FWER-corrected p<0.05, cluster threshold 5. Coordinates are reported in mm in MNI space.

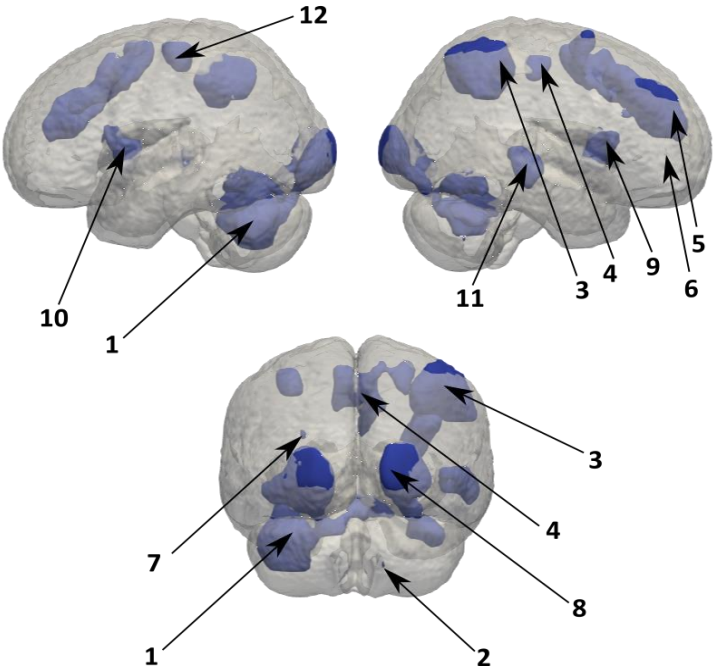


Figure III: Neural activity when inhibiting at No-Go cues (n=83) (FWER correction at cluster level, p<0.05).

1: Left anterior part of cerebellum, 2: Right anterior part of cerebellum, 3: Right inferior parietal region, 4: Medial cingular region, 5: Right cingular region, 6: Right frontal region, 7: Left frontal region, 8: Right occipital region (inferior/middle), 9: Right insular region, 10: Left insular region, 11: Right temporal region, 12: Left pre/post-central region

Table 5: Neural activity associated with incorrect answers at No-Go cues (n=83) (FWER correction at cluster level, p<0.05).

Cluster			**Cluster foci**			
Structure	**Hemisphere**	**Volume (in mm^3)**	**X**	**Y**	**Z**	**t-value**
SMA	R/L	84576	4	20	36	12.46
Middle cingulate	R/L	1152	2	-20	32	6.27
Inferior frontal region	L	7408	-26	44	24	7.30
Inferior parietal region	R	18192	50	-54	48	10.78
	L	4496	-58	-54	36	6.68
Insular region (Insula, inferior frontal)	R	11968	32	16	-12	8.96
	L	9568	-30	16	-12	9.13
	L	80	-30	50	0	4.85
Anterior part of cerebellum	L	9104	-36	-54	-32	9.55
	R	2240	38	-52	-32	8.00
Temporal region (middle, inferior)	R	6336	50	-28	-4	7.99
	R	1040	50	0	-36	6.09
	L	672	-52	0	-36	5.90
Occipital region	R	1120	18	-104	4	7.44
	L	1080	-16	-104	0	5.51
Thalamus	R	432	10	-6	8	5.41

Cluster volume is reported in mm^3. Cluster foci of highest t-value at FWER-corrected p<0.05, cluster threshold 5. Coordinates are reported in mm in MNI space.

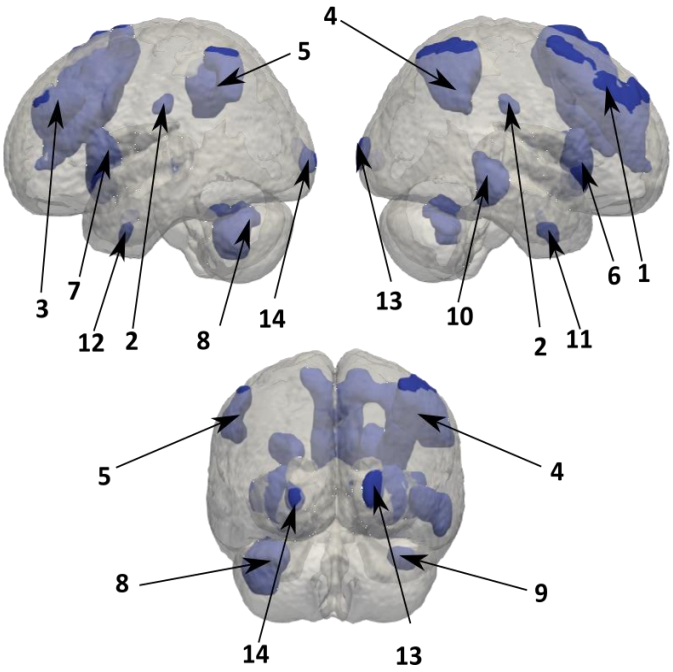


Figure IV: Neural activity associated with incorrect answers at No-Go cues (n=83) (FWER correction at cluster level, p<0.05).

1, 2: Medial cingular regions, 3: Left frontal region, 4: Right inferior parietal region, 5: Left inferior parietal region, 6: Right insular region, 7: Left insular region, 8: Left anterior part of cerebellum, 9: Right anterior part of cerebellum, 10, 11: Right temporal region, 12: Left temporal region, 13: Right occipital region, 14: Left occipital region

Table 6: Neural activity associated with "Successful No-Go vs successful Go" contrast (n=83) (FWER correction at cluster level, p<0.05).

Cluster			**Cluster foci**			
Structure	**Hemisphere**	**Volume (in mm³)**	**X**	**Y**	**Z**	**t-value**
Anterior part of cerebellum	L	14944	-38	-56	-44	9.64
	R	800	40	-56	-28	5.95
Supramarginal gyrus	R	14464	42	-46	56	10.50
Frontal region (middle, sup)	R	9968	34	50	24	8.37
	R	1680	28	-2	60	7.73
	R	3024	16	12	-4	6.66
	R	320	40	12	40	5.23
Putamen	L	1712	-22	8	0	6.69
Inferior frontal gyrus	L	304	-32	30	24	5.46
Insula	L	144	-38	12	4	5.46
Cingular region (SMA, ant/middle cingular)	R/L	2976	4	28	32	6.41
Temporal region	R	2768	58	-24	-4	6.46
Occipital region	L	1792	-18	-100	12	6.02
	L	1056	-38	-90	-4	6.15
	R	720	16	-102	4	6.06
Thalamus	L	80	-8	-4	4	5.52

Cluster volume is reported in mm³. Cluster foci of highest t-value at FWER-corrected p<0.05, cluster threshold 5. Coordinates are reported in mm in MNI space.

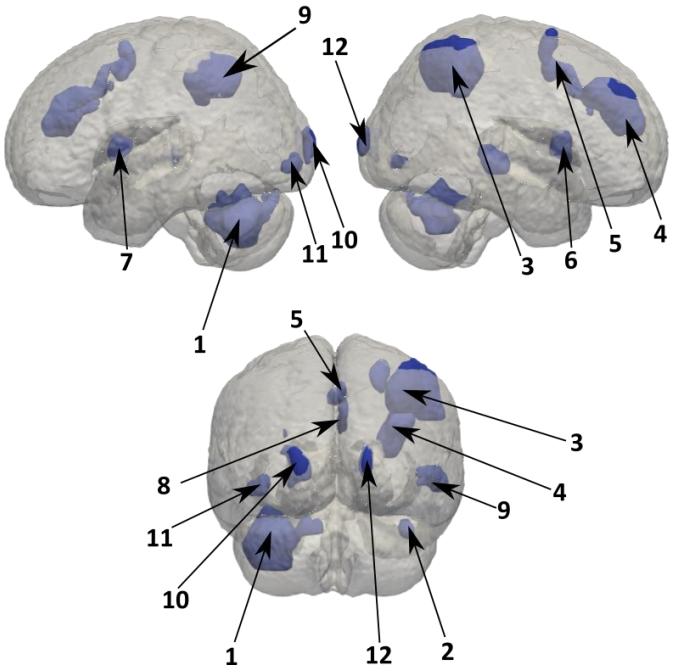


Figure V: Neural activity associated with "Successful No-Go vs successful Go" contrast (n=83) (FWER correction at cluster level, p<0.05).

1: Left anterior part of cerebellum, 2: Right anterior part of cerebellum, 3: Right supramarginal gyrus, 4: Right inferior frontal regions 5:Right superior frontal regions, 6: Right insular region, 7: Left insular region, 8: Cingular region, 9: Right temporal region, 10, 11: Left occipital regions, 12: Right occipital region

Table 7: Neural activity associated with "No-Go vs successful Go" contrast (n=83) (FWER correction at cluster level, p<0.05).

Cluster			**Cluster foci**			
Structure	**Hemisphere**	**Volume (in mm³)**	**X**	**Y**	**Z**	**t-value**
Anterior cingulate	R/L	77280	4	28	32	13.07
Middle cingulate	R/L	1136	4	-22	32	6.54
Supramarginal gyrus	R	19696	48	-42	44	11.28
	L	4448	-58	-54	36	6.95
Insula	R	11808	32	16	-12	9.67
	L	9424	-32	16	-12	9.87
Anterior part of cerebellum	L	8960	-36	-54	-32	9.67
	R	1504	38	-52	-32	7.31
Middle frontal region	L	7440	-38	42	28	8.60
	L	80	-30	48	4	5.12
Middle temporal region	R	5712	48	-30	-4	8.03
	R	832	50	0	-32	5.92
	L	544	-54	0	-32	5.51
Occipital region	R	240	16	-104	4	5.77
Thalamus	R	320	8	-16	8	5.78

Cluster volume is reported in mm³. Cluster foci of highest t-value at FWER-corrected p<0.05, cluster threshold 5. Coordinates are reported in mm in MNI space.

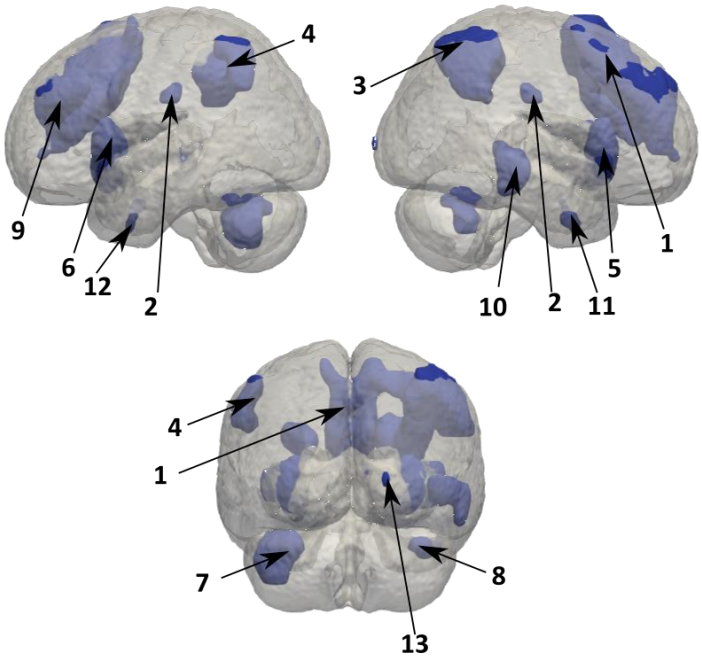


Figure VI: Neural activity associated with "No-Go vs successful Go" contrast (n=83) (FWER correction at cluster level, p<0.05).

1, 2: Cingular region, 3: Right inferior parietal region, 4: Left inferior parietal region, 5: Right insular region, 6: Left insular region, 7: Left anterior part of cerebellum, 8: Right anterior part of cerebellum, 9: Left frontal region, 10, 11: Right temporal region, 12: Left temporal region, 13: Right occipital region

Table 9: Correlations between activations of successful Go contrast and neuropsychological scores (n=83).

Cluster		Omissions	Response latency	Visual attention	Auditory attention	Response set
Pre/post-central region	L	0.02	0.02	-0.03	0.01	-0.18
Occipital region (inferior/middle/sup)	R L	0.12 0.12	-0.06 -0.14	0.07 0.15	0.18 0.01	0.06 0.01
Supplementary motor area (SMA)	L	-0.16	-0.05	0.03	0.06	0.10
Anterior part of cerebellum	R R	-0.03 -0.12	-0.18 -0.18	0.14 0.10	0.06 0.01	-0.02 0.12
Medial superior frontal region Inferior parietal region	R R	-0.08 -0.05	0.09 0.20†	-0.07 0.03	0.03 0.00	0.00 -0.05

*Spearman coefficients. * : p<0.05, †: 0.05≤ p <0.10*

Table 10: Correlations between activations of Successful No-Go vs successful Go contrast and neuropsychological scores (n=83).

Cluster		**Commissions**	**Visual attention**	**Auditory attention**	**Response set**
Anterior part of cerebellum	L	0.07	0.15	0.05	0.16
	R	0.07	0.03	-0.09	0.10
Inferior parietal region	R	0.17	0.12	0.07	0.07
Frontal region (middle, sup)	R	0.00	0.13	0.13	0.06
	R	0.02	0.13	0.04	-0.05
	R	0.01	0.11	0.07	0.10
	R	-0.04	0.09	0.15	-0.01
Insula	L	0.21†	0.23*	0.12	0.13
	L	0.09	0.24*	0.09	0.09
	L	0.26*	-0.01	0.03	-0.06
Cingular region (SMA, ant/middle cingular)	R/L	0.17	0.14	-0.10	0.07
Temporal region	R	-0.06	0.18	-0.07	-0.03
Occipital region	L	0.14	0.07	-0.02	-0.07
	L	0.02	-0.01	-0.07	0.14
	R	0.16	-0.04	-0.06	0.02
Thalamus	L	-0.07	0.10	0.11	0.12

*Spearman coefficients. *: p<0.05, †: 0.05≤ p <0.10*

Table 11: Correlations between activations of No-Go vs Go contrast and neuropsychological scores (n=83).

Cluster		**Commissions**	**Visual attention**	**Auditory attention**	**Response set**
Anterior cingulate	R/L	-0.31*	0.23*	0.23*	0.21†
Middle cingulate	R/L	-0.28*	0.11	0.10	0.29*
Supramarginal gyrus	R	-0.18	0.15	0.15	0.13
	L	-0.20†	0.09	0.16	0.18
Insula	R	-0.25*	0.15	0.24*	0.13
	L	-0.21†	0.12	0.13	0.17
Anterior part of cerebellum	L	0.05	0.06	0.10	0.13
	R	0.09	-0.15	0.08	0.26*
Middle frontal region	L	-0.19†	0.02	-0.02	-0.05
	L	-0.18	0.18	0.14	0.09
Middle temporal region	R	-0.21†	0.14	0.11	0.07
	R	-0.16	0.07	0.17	0.19†
	L	-0.15	-0.04	0.13	0.04
Occipital region	R	0.16	-0.01	0.05	0.07
Thalamus	R	-0.08	0.05	0.03	0.11

*Spearman coefficients. *: p<0.05, †: 0.05≤ p <0.10*

References

1. Bari A, Robbins TW. Inhibition and impulsivity: behavioral and neural basis of response control. Progress in Neurobiology. 2013 Sep 1;108(Supplement C):44–79.

2. Willcutt EG, Doyle AE, Nigg JT, Faraone SV, Pennington BF. Validity of the Executive Function Theory of Attention-Deficit/Hyperactivity Disorder: A Meta-Analytic Review. Biological Psychiatry. 2005 Jun 1;57(11):1336–46.

3. Diamond A, Barnett WS, Thomas J, Munro S. Preschool Program Improves Cognitive Control. Science. 2007 Nov 30;318(5855):1387–8.

4. Moffitt TE, Arseneault L, Belsky D, Dickson N, Hancox RJ, Harrington H, et al. A gradient of childhood self-control predicts health, wealth, and public safety. PNAS. 2011 Feb 15;108(7):2693–8.

5. Rubia K, Russell T, Overmeyer S, Brammer MJ, Bullmore ET, Sharma T, et al. Mapping Motor Inhibition: Conjunctive Brain Activations across Different Versions of Go/No-Go and Stop Tasks. NeuroImage. 2001 Feb;13(2):250–61.

6. Simmonds DJ, Pekar JJ, Mostofsky SH. Meta-analysis of Go/No-go tasks demonstrating that fMRI activation associated with response inhibition is task-dependent. Neuropsychologia. 2008 Jan 15;46(1):224–32.

7. Lütcke H, Frahm J. Lateralized anterior cingulate function during error processing and conflict monitoring as revealed by high-resolution fmri. Cerebral Cortex. 2008 Mar;18(3):508–15.

8. Borst G, Cachia A, Vidal J, Simon G, Fischer C, Pineau A, et al. Folding of the anterior cingulate cortex partially explains inhibitory control during childhood: A longitudinal study. Developmental Cognitive Neuroscience. 2014 Jul;9:126–35.

9. Garlantézec R, Monfort C, Rouget F, Cordier S. Maternal occupational exposure to solvents and congenital malformations: a prospective study in the general population. Occupational and Environmental Medicine. 2009 Jul 1;66(7):456–63.

10. Mostofsky SH, Schafer JG, Abrams MT, Goldberg MC, Flower AA, Boyce A, et al. fMRI evidence that the neural basis of response inhibition is task-dependent. Cognitive brain research. 2003;17(2):419–430.

11. Ashburner J. A fast diffeomorphic image registration algorithm. NeuroImage. 2007 Oct;38(1):95–113.

12. Korkman M, Kirk U, Kemp S. NEPSY [: A Developmental Neuropsychological Assessment. Psychological Corporation; 1998.

GLYCOL ETHERS AND MOTOR INHIBITION

Status: published in *Environment international*

Binter AC, Bannier E, Saint-Amour D, Simon G, Ferré JC, Barillot C, Monfort C, Cordier S, Chevrier C, Pelé F. Prenatal exposure to glycol ethers and motor inhibition function evaluated by functional MRI at the age of 10 to 12 years in the PELAGIE mother-child cohort. *Environ Int. sep 2019;133:105163.*

2.1. Résumé

Contexte : Les femmes enceintes sont fréquemment exposées aux solvants organiques, tels que les éthers de glycol. Plusieurs études suggèrent une potentielle toxicité de l'exposition prénatale aux éthers de glycol sur le cerveau en développement mais les possibles mécanismes neuronaux restent mal définis.
Objectifs : Nous étudions l'association entre les niveaux d'éthers de glycol dans les urines de femmes en début de grossesse et la fonction inhibitrice de leurs enfants âgés de 10 à 12 ans évaluées par des tests comportementaux et par imagerie cérébrale.
Méthodes : L'exposition aux éthers de glycol est estimée en mesurant six métabolites urinaires de 73 femmes enceintes (avant 19 semaines de gestation) recrutées dans la cohorte mère-enfant PELAGIE (France). Les niveaux urinaires sont classés en faibles, modérés, élevés. Les enfants ont passé un examen d'imagerie par résonance magnétique fonctionnelle (IRMf) durant lequel la fonction d'inhibition motrice est évaluée par une tâche de Go/No-Go. Les analyses statistiques sont réalisées en utilisant des modèles de régression linéaire pour les performances à la tâche et des modèles linéaires généralisés à effets mixtes pour les activations cérébrales, corrigés pour tests multiples au niveau des clusters (correction FWER). Les facteurs de confusion sont pris en compte par restriction à l'inclusion ou par ajustement des modèles.
Résultats : Les concentrations urinaires d'acide butoxyacétique (BAA) les plus élevées étaient associées à de plus faibles performances chez l'enfant (β = – 1.1; intervalle de confiance à 95% (IC95%) : – 1.9, – 0.2 for high vs low). Nous avons également observé une tendance à de plus faibles performances en lien avec l'acide éthoxyacétique (EAA) (β = – 0.3; IC95% : – 0.7, 0.01). En considérant la consigne d'inhibition, l'activité dans les régions occipitales augmente en association avec des niveaux modérés d'EAA (cuneus gauche) et d'acide méthoxyacétique (MAA) (precuneus droit). Lorsque les enfants réussissaient à inhiber, les niveaux élevés d'acide ethoxyethoxyacétique (EEAA) et modérés d'acide phénoxyacétique (PhAA) étaient associés avec des activités différentielles (diminution et augmentation, respectivement) dans le cortex frontal, une région cérébrale impliquée dans le réseau de l'in-

hibition.
Discussion : Les niveaux urinaires prénataux de deux métabolites d'éthers de glycol ont été associés à de plus faibles performances à la tâche de Go/No-Go. Des activations différentielles ont été observées dans les régions du cerveau impliquées dans l'inhibition en cas d'inhibition réussie mais pas en considérant la demande d'inhibition. Il n'y a cependant pas de cohérence entre les indicateurs de performances et les résultats sur les activations cérébrales. D'autres études sont nécessaires en raison du caractère ubiquitaire de l'exposition aux éthers de glycol.

2.2. Prenatal exposure to glycol ethers and motor inhibition

The article resulting from the study is inserted on the next pages.

Environment International 133 (2019) 105163

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Prenatal exposure to glycol ethers and motor inhibition function evaluated by functional MRI at the age of 10 to 12 years in the PELAGIE mother-child cohort

Anne-Claire Binter[a,*], Elise Bannier[b,e], Grégory Simon[c], Dave Saint-Amour[d], Jean-Christophe Ferré[b,e], Christian Barillot[b], Christine Monfort[a], Sylvaine Cordier[a], Cécile Chevrier[a], Fabienne Pelé[a,f]

[a] Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR_S 1085, F-35000 Rennes, France
[b] Univ Rennes, CNRS, Inria, Inserm, IRISA UMR 6074, VISAGES (Vision, action et gestion des informations en santé) - ERL U 1228, F-35000 Rennes, France
[c] ISTS EA 7466, University of Caen Normandie, Caen, France
[d] Department of Psychology, Université du Québec à Montréal, Montréal, Canada
[e] CHU Rennes, Radiology Department, Rennes, France
[f] Univ Rennes, Inserm, CIC 1414, Rennes, France

ARTICLE INFO

Handling Editor: Martí Nadal

ABSTRACT

Background: Pregnant women are ubiquitously exposed to organic solvents, such as glycol ethers. Several studies suggest potential developmental neurotoxicity following exposure to glycol ethers with a lack of clarity of possible brain mechanisms.

Objectives: We investigated the association between urinary levels of glycol ethers of women during early pregnancy and motor inhibition function of their 10- to 12-year-old children by behavioral assessment and brain imaging.

Methods: Exposure to glycol ethers was assessed by measuring six metabolites in urine (< 19 weeks of gestation) of 73 pregnant women of the PELAGIE mother-child cohort (France). Maternal urinary levels were classified as low, medium, or high. Children underwent functional magnetic resonance imaging (fMRI) examinations during which motor inhibition function was assessed with a Go/No-Go task. Analyses were performed using linear regression for task performance and generalized linear mixed-effect models for brain activation, FWER-corrected for multiple testing at the spatial cluster level. Confounders were considered by restriction and *a priori* adjustment.

Results: Higher maternal butoxyacetic acid (BAA) urinary concentrations were associated with poorer child performance ($\beta = -1.1$; 95% CI: -1.9, -0.2 for high *vs* low). There was also a trend for ethoxyacetic acid (EAA) towards poorer performance ($\beta = -0.3$; 95% CI: -0.7, 0.01). Considering inhibition demand, there were increased activity in occipital regions in association with moderate EAA (left cuneus) and moderate methoxyacetic acid (MAA) (right precuneus). When children succeeded to inhibit, high ethoxyethoxyacetic acid (EEAA) and moderate phenoxyacetic acid (PhAA) levels were associated with differential activity in frontal cortex, involved in inhibition network.

Discussion: Prenatal urinary levels of two glycol ether metabolites were associated with poorer Go/No-Go task performance. Differential activations were observed in the brain motor inhibition network in relation with successful inhibition, but not with cognitive demand. Nevertheless, there is no consistence between performance indicators and cerebral activity results. Other studies are highly necessary given the ubiquity of glycol ether exposure.

* Corresponding author at: Inserm UMR 1085 Irset, 9 Avenue du Pr. Léon Bernard, 35000 Rennes, France.
E-mail address: anne-claire.binter@inserm.fr (A.-C. Binter).

https://doi.org/10.1016/j.envint.2019.105163
Received 16 January 2019; Received in revised form 8 August 2019; Accepted 5 September 2019
0160-4120/ © 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

1. Introduction

The use of organic solvents is widespread in industries, mainly to dissolve other substances without altering them or themselves. Although glycol ethers represent only 4% of solvent consumption in France, pregnant women are ubiquitously exposed to these chemicals according to French and European biomonitoring data (AFSSET, 2008; Fromme et al., 2013; Garlantézec et al., 2013; Béranger et al., 2017). For instance, metabolites of glycol ethers have been detected in at least 90% of urine samples of pregnant women in the PELAGIE cohort (2002–2006) (Béranger et al., 2017). Glycol ethers are oxygenated solvents which are highly miscible in oil and water. Their use is authorized in both occupational and domestic products and they are present in various products, such as paint, varnish, and ink, as well as cosmetics and agrochemicals. There are several possible routes of exposure (dermal, pulmonary or oral), depending on their formulation and context of use (AFSSET, 2008).

Organic solvents can pass the placental barrier and the developing blood-brain barrier (Grandjean and Landrigan, 2006). The developing brain is sensitive to its environment and solvent exposure during critical growth phases could result in neurobehavioral effects later in life (Julvez and Grandjean, 2009a; Rice and Barone, 2000). Several studies have investigated the association between prenatal exposure to solvents and the neurodevelopment of children. In an occupational setting, Eskenazi et al. did not find any association between prenatal solvent exposure and child behavior at 3.5 years of age, but exposed children (n = 92) started walking slightly earlier (Eskenazi et al., 1988). A Canadian study reported lower language scores and graphomotor abilities in exposed three- to seven-year-old children (n = 61), suggesting impaired cognitive and neuromotor function (Till et al., 2001a,b). Nevertheless, in 2004, a similar study of three- to nine-year-old children (n = 64) showed an association between prenatal solvent exposure and cognitive (processing speed, memory), motor (dexterity and visual-motor coordination), and behavioral (hyperactivity/impulsivity) scores (Laslo-Baker et al., 2004). In 2005, Till et al. reported reduced visual acuity in infants prenatally exposed to solvents (n = 59) (Till et al., 2001a,b; Till et al., 2005). In 2013, Pelé et al. investigated effects of occupational exposure to solvents during pregnancy and child behavior at two years of age in a population-based cohort (n = 1278) (Pelé et al., 2013). Women were asked to report the frequency (never, occasional, regular) of occupational contact with products known to contain solvents and classified as occasionally or regularly exposed if they reported occasional or regular exposure, respectively, to at least one product. They observed an association between occasional and regular solvent exposure at work and higher attention deficit/hyperactivity and aggression scores. These studies all investigated exposure to solvents in occupational settings for which levels of exposure are likely to be higher than that of the general population. In addition, these studies dealt with different solvent mixtures (mainly petroleum or halogenated solvents, except for that of Pelé et al. in which women were mainly exposed to oxygenated solvents). In 2017, Beranger et al. investigated the specific effects of glycol ethers on neurodevelopment in a population-based mother-child cohort. The authors reported that six-year-old children with the highest level of *in-utero* exposure to glycol ethers had lower verbal comprehension and copying scores (n = 204) (Béranger et al., 2017). These findings are original and supported by only a few animal studies on rats, suggesting that learning ability and neurochemistry are affected after prenatal exposure to low levels of 2-methoxyethanol or 2-ethoxyethanol (Nelson et al., 1984; Nelson and Brightwell, 1984).

Apart from Till et al. and Eskenazi et al., these studies all reported learning disabilities or behavioral alterations. These cognitive processes are supported by executive functions, in particular inhibitory control (Bari and Robbins, 2013; Diamond et al., 2007). Inhibitory control is critical for learning ability (Bari and Robbins, 2013) and difficulties in the ability to control impulses and inhibit a prepotent response are related to various conditions, such as attention deficit hyperactivity disorder (Willcutt et al., 2005) and poor academic performance (Diamond et al., 2007). In healthy subjects, the efficiency of inhibition during childhood is a strong predictor of future academic and professional outcomes (Moffitt et al., 2011).

In light of the adverse effects observed in children, we investigated potential effects on inhibitory control in a general population-based cohort and look further into possible neural underpinnings suggested by the literature. We hypothesize that investigating functional brain processes while children are engaged in specific cognitive tasks suspected to be affected by glycol ethers could provide finer tools.

Thus, we aimed to investigate the suspected neurotoxicity of glycol ethers after prenatal exposure on the inhibitory control of 10- to12-year-old children from a population-based mother-child cohort using motor inhibition task during functional magnetic resonance imaging (fMRI) and neuropsychological tests.

2. Material and methods

2.1. Population

The PELAGIE (*Perturbateurs endocriniens: Etude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance*) mother-child cohort included 3421 women at the beginning of pregnancy (before 19 weeks of gestation) from three districts of Brittany (France) between 2002 et 2006 (Garlantézec et al., 2009). They were recruited from the general population by obstetricians, gynecologists, and ultra-sonographers at early visits for prenatal care. At inclusion, they completed a self-administered questionnaire about family, social, and demographic characteristics, diet, and lifestyle.

A sub-cohort of 265 children between 10 and 12 years of age was randomly selected for the present study to include 100 children, since we expected a participation rate below 50%. We actually invited 251 families. Children had to 1. be live-born singleton, 2. be delivered after 35 weeks of amenorrhea, 3. present no major condition at birth (neonatal hospitalization, hypoglycemia, five-minute Apgar score < 7) (Cartier et al., 2016), 4. present no prenatal exposure to tobacco, alcohol, or medical treatment during childhood which could affect neurodevelopment (methylphenidate, psychotropic or antiepileptic drugs, *etc.*) and 5. have a maternal urine sample collected at inclusion (minimum–maximum, 4–17; median, 10 weeks; interquartile range, 8–11) available for chemical analysis. Among the 251 families, 124 (46.4%) refused to participate or were lost to follow-up, 26 (9.8%) were excluded due to technical (braces) or medical reasons (meningitis, head trauma). Thus, 101 (38.1%) children participated in neuropsychological and functional MRI examinations.

Testing took place at the Clinical Investigation Unit of the Rennes University Hospital from November 2014 to November 2016. They were performed on Wednesdays out the French school holidays periods or during the all days of the week except week-end during holidays. Proceedings were standardized as much as possible: all MRI sequences were carried out at the beginning of the testing for almost two hours (45 minute-training and 1 h in the MRI scanner).

Urine samples were not available for 28 women because their samples were used for other urinary assays, resulting in 73 mother-child pairs (72.3%) for our study population (see Supplementary material, Fig. S.I). Parents completed a questionnaire about the environmental and health conditions of their child.

All parent and child participants provided written informed consent and the appropriate ethics committees approved the study (Committee for the Protection of Persons- n°2013-A01420-45, French Consulting Committee for the Treatment of Information in Medical Research, n°09.485, and the French National Commission for the Confidentiality of Computerized Data, n°909347).

Table 1
Alkoxycarboxylic acids measured in urine samples, precursors, and sources (AFSSET, 2008).

Alkoxycarboxylic acid (metabolite)	Glycol ethers (parent compound)	Sources
MAA	EG(D)ME, DEG(D)ME, TEG(D)ME	Cleaning agents
EAA	EG(D)EE, DEG(D)EE, TEGEE	Biocides, cleaning agents, cosmetics, drugs, paints
EEAA	DEGEE, TEGEE	Biocides, cleaning agents, cosmetics, drugs, paints
BAA	EGBE, DEGBE, TEGBE	Biocides, cleaning agents, cosmetics, paints
PhAA	EGPhE	Biocides, cosmetics, drugs
2-MPA	1PG2ME (β isomer)	Biocides, cleaning agents, paints

Abbreviations: BAA, 2-butoxyacetic acid; DEGBE, 2-(2-butoxyethoxy)ethanol; DEGDEE, 2-(2-Ethoxyethoxy)-1-ethoxyethane; DEGDME, 2-(2-Methoxyethoxy)-1-methoxyethane; DEGEE, 2-(2-ethoxyethoxy)ethanol; DEGME, 2-(2-methoxyethoxy)ethanol; EAA, ethoxyacetic acid; EEAA, ethoxyethoxyacetic acid; EGBE, 2-butoxyethanol; EGDEE, 1,2-Diethoxyethane; EGDME, 1,2-Diethoxymethane; EGEE, 2-ethoxyethanol; EGME, 2-methoxyethanol; EGPhE, 2-phenoxyethanol; MAA, methoxyacetic acid; 2-MPA, 2-methoxyproprionic acid; 1PG2ME, methoxy-propanol; PhAA, phenoxyacetic acid; TEGBE, 2-(2-(2-Butoxyethoxy)ethoxy)ethanol; TEGDME, 1,2-bis(methoxyethoxy)ethane; TEGEE, 2-(2-(2-ethoxyethoxy)ethoxy)ethanol; TEGME, 2-(2-(2-methoxyethoxy) ethoxy)ethanol.

2.2. Solvent exposure assessment

At inclusion, pregnant women returned a urine sample (first morning void) in a 10-mL test tube (95 × 16-mm polypropylene, with wing plug). Six alkoxycarboxylic acids (glycol ether metabolites) of the most commonly used glycol ethers in France at the time of inclusion (Table 1) were analyzed: methoxyacetic acid (MAA), ethoxyacetic acid (EAA), ethoxyethoxyacetic acid (EEAA), 2-butoxyacetic acid (BAA), phenoxyacetic acid (PhAA), and 2-methoxypropionic acid (2-MPA). Analyses were performed in 2013 (n = 54) and 2017 (n = 19) by gas chromatography–mass spectrometry (GC–MS) at the LABOCEA (Laboratoire public Conseil, Expertise et Analyse en Bretagne) with a limit of detection (LD) of 3 µg/L. Details of the chemical analysis procedures are described elsewhere (Béranger et al., 2017). We found no mean differences between the two series of measurements (see Supplementary material, Fig. S.II and Table S.1). Glycol ether metabolite levels were categorized into three groups. For clarity, we refer hereafter to low, moderate, or high levels. Values below the LD were imputed from a lognormal distribution (Jin et al., 2011).

2.3. Inhibition function

2.3.1. Task

Children completed a 10-minute task in the MR scanner. First, short practices were performed in front of a computer then in a mock MR scanner to provide the instructions and accustom the child to the scanner environment.

Motor inhibition function of the children was evaluated with a visual Go/No-Go task. This event-related task, adapted from Mostofsky et al. (2003), minimizes cognitive demands other than motor execution and response inhibition and can be easily performed in children (Réveillon et al., 2013; Suskauer et al., 2008). Green and red smileys were successively presented on a screen. Children were asked to press a button as quickly as possible when seeing a green smiley but had to refrain otherwise. The ratio of No-go cues (red smileys) over Go cues (green smileys) was 1:4, to elicit a dominant response. The task was implemented using *E*-Prime v.2.0.8 Professional (Psychology Software Tools, Pittsburgh, PA, USA) and presented using the Nordic Neurolab Solution (Nordic Neurolab, Bergen, Norway).

The task was split into two runs of 5 min each. Each run consisted of 150 trials and four 10-second rest periods. A trial was the consecutive presentation of a smiley (duration of 200 ms) and a cross fixation point (duration of 1300 ms). A response was allowed until the next trial began, giving a constant inter-stimulus interval (ISI) of 1500 ms. Smileys appeared in a pseudo-random order: trials at the beginning of runs or following resting periods had to be Go cues. Go cues had to occur at least three times in a row and No-Go cues had to occur individually or at most two times in a row.

2.3.2. Performance

Child performance for the Go/No-Go task was evaluated by response latency (RLs, average reaction time for the correct answering of Go cues), commission rate (incorrect answers for the No-Go cues), and performance score (PS). Responses before 200 ms were considered to be anticipatory and were excluded from the indicator calculation (on average, < 1% of all cues). The accuracy of motor inhibition was assessed by the sensitivity index, *d′*, which subtracts the standardized (z score) commission rate from the standardized hit rate (correct answers for Go cues). The RLs and commission errors inversely correlated with each other (Spearman rho coefficient = −0.32, p = 0.01). Thus, we built a PS by subtracting the RLs from the accuracy of motor inhibition (*d′*), following standardization (Collignon et al., 2010).

$$d' = Z(\textit{hit rate}) - Z(\textit{commission rate})$$
$$PS = Z(d') - Z(RLs)$$
$$(Z\text{: standardized value})$$

Thus, children with the highest PS scores were considered to efficiently perform the task (fast with a high hit rate, with few commissions), whereas children with the lowest PS scores were slow and found it difficult to inhibit their response to No-Go cues. Performance indicators could not be recorded for one child (n = 72), due to technical issues with the recording.

2.3.3. Cerebral hemodynamic response related to the task

Neural activity related to motor inhibition was evaluated indirectly by blood oxygen-level dependent (BOLD) imaging.

Scanning was completed on a 3 T MR Scanner (Magnetom Verio, VB17, Siemens, Erlangen, Germany) using a 32-channel receiver head coil. Functional images were acquired using gradient echo-planar imaging (EPI) with repetition/echo times of 2500/30 ms, a 90° flip angle, and 110 volumes per run. Each volume was composed of 34 axially oriented 4 mm-interleaved slices, covering the whole brain. Scans with a voxel size of 2*2*4 mm^3 were based on a 110*110 acquisition matrix (220 × 220 mm^2 field of view (FOV)). Operators assessed image quality during the acquisition, two children who moved repeated the sequence. Learning effects are not expected for this task and we did not observe any improvement in performance during the two sessions.

High-resolution 3D anatomical images were obtained using T1-weighted MPRAGE at 1 mm^3 resolution for anatomical referencing (repetition/inversion/echo times: 1900/900/2.26 ms, 9° flip angle).

Image pre-processing is described in Supplementary material. Head movements were evaluated by six parameters calculated during the realignment step. Two children had head motion > 2 mm (in-plane size of an acquisition voxel) and their images were excluded from further analysis (n = 71).

The hemodynamic response was modeled from trials (Go cue and No-Go cue), implicit baseline (resting periods enabling recovery of the hemodynamic response and the time between trials), and motion

regressors using a canonical hemodynamic response function (HRF) and its temporal derivative. This HRF is the subtraction of two gamma functions. The first gamma function models the peak of intensity with a latency of 6 s and the second the undershoot during the recovery period with a latency of 16 s. The use of a temporal derivative allows for variations in peak latency, while providing comprehensive models for the response. Scanner drift was modeled with a discrete cosine transform (DCT) set (128-second cut off) and temporal autocorrelation was accounted for using an autoregressive AR(1) model over the whole brain. First, we modeled Go cue and No-Go cue, independently of the subject's response. Secondly, we modeled successful No-Go, failed No-Go and Go conditions. Maps were extracted for each condition and for each subject by voxel-wise multiple regressions estimated by the restricted maximum likelihood (ReML) method and then contrasted. Because we were investigating the ability to stop a planned response when it is no longer pertinent, we built two types of individual contrast images. First, we extracted "No-Go vs Go" activations, representing activation amplitudes that were higher for inhibition than motor tasks, when children perceived the inhibition demand. Secondly, we built contrast images for "Successful No-Go vs successful Go", when children were able to inhibit and stop their answers. Results for failed inhibition contrast "Failed No-Go vs successful Go" were reported for information (see Supplementary material, Table S.6).

There were no differences between the two runs for contrast estimate (at the uncorrected cluster level $p < 0.001$) or any performance indicators (at $p < 0.05$), allowing concatenation of the two runs.

2.4. Analysis strategy and statistical analyses

Multivariable linear regressions were built on performance indicators (log-transformed-reaction time, commission rate, and PS) to analyze the association with each categorical level of glycol ether metabolite. We used restricted cubic splines with log-transformed metabolite concentrations to assess a possible dose-response relation. When the assumption of linearity was assessed, we plotted urinary metabolite levels as continuous and reported their p-values as a trend test.

The hemodynamic response during the motor inhibition task was modeled by mixed effect generalized linear regressions to investigate the interaction between the category of prenatal exposure to glycol ethers and the effects of the motor inhibition task on cerebral activity. We did not have any *a priori* hypotheses on brain regions that could be differentially activated during the inhibition task across exposure levels, so we performed whole-brain analyses. Some regions are known to be part of the motor inhibition network (frontal and anterior cingular cortices and supplementary motor area (SMA)) regions (Mostofsky et al., 2003; Suskauer et al., 2008). Thus, we highlighted our results for these regions. Statistical significance was assessed for cluster-wise significance, defined by random field theory (FWER corrected $p = 0.05$) to account for 3D spatial autocorrelation, using an uncorrected one-sided cluster-defining threshold of $p = 0.01$ (Nichols and Hayasaka, 2003).

Maternal educational level ($<$ *vs* ≥ 3 years of post-secondary school attendance) and breastfeeding of the child ($\leq$ *vs* > 3 months) were included *a priori* in all regression models as potential confounders. A missing covariate value (maternal educational level, n = 1) was imputed by the mode of the distribution.

R software v.3.4.1 (https://www.R-project.org/) and SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) were used for data analysis.

3. Results

3.1. Population characteristics

Participant characteristics are presented in Table 2. Women included in the study had a median age of 30.4 years at inclusion. This population was highly educated (45.8% attended at least three years of post-secondary school) and reported few medical problems during pregnancy (2.7% reported high blood pressure, no diabetes). The children's median birth weight was 3430 g, 43.8% were breastfed for more than three months, and 38.4% were boys. Children attending follow-up were mainly right-handed (87.7%), attended mainly elementary school (74.0%) and had a median age of 10.8 years. We did not observe any differences for these characteristics between the study population and non-participants (see Supplementary material, Table S.2).

Table 2
Characteristics of the population (n = 73).

Maternal and child characteristics		N (%)	Median (Q1;Q3)
Maternal characteristics (at inclusion)			
Age (in years)			30.4 (28.4;33.5)
Body Mass Index before pregnancy	(< 18.5 kg/m^2)	9 (12.3)	
	(18.5–25 kg/m^2)	51 (69.9)	
	(≥ 25 kg/m^2)	13 (17.8)	
Post-secondary school	(≤ 2 years)	39 (54.2)	
	(≥ 3 years)	33 (45.8)	
	Unknown	1	
High blood pressure (gestational or not; yes)		2 (2.7)	
Diabetes (gestational or not; yes)		0 (0)	
Subject characteristics			
Sex (boys)		28 (38.4)	
Gestational age (in weeks of amenorrhea)			40 (39;40)
Birth weight (in g)			3430 (3180;3800)
Breastfeeding (> 3 months)		32 (43.8)	
Parity	(single child)	10 (13.7)	
	(1 sibling)	35 (47.95)	
	(≥ 2 siblings)	28 (38.36)	
Age (in years)			10.8 (10.6;11)
Educational level	(Elementary school)	54 (74.0)	
	(Junior high school)	19 (27.0)	
Lateralization (right-hander)		64 (87.7)	

3.2. Urinary glycol ether metabolite levels

Each glycol ether metabolite was detected in the urine of at least 89% of the women, with median values from 15 (for EAA) to 275 µg/L (for PhAA) (Table 3). There were slight correlations between MAA and 2-MPA concentrations (Spearman rho coefficient = 0.32, p = 0.02) and PhAA and EEAA (rho = 0.17, p = 0.04) (see Supplementary material, Fig. S.II). EEAA, EAA, and 2-MPA levels also correlated with each other (EAA-EEAA, rho = 0.36; EAA-2-MPA, rho = 0.31; EEAA-2-MPA,

Table 3
Detection rates and median concentrations of glycol ether metabolites in maternal urine (n = 73). Abbreviations: BAA, 2-butoxyacetic acid; EAA, ethoxyacetic acid; EEAA, ethoxyethoxyacetic acid; MAA, methoxyacetic acid; 2-MPA, 2-methoxyproprionic acid; PhAA, phenoxyacetic acid.

Glycol ether metabolites	N (%) > LD	Median (µg/L) (1st tercile;2nd tercile)[a]	Max (µg/L)
MAA	71 (97.3%)	63 (52;83)	220
EAA	66 (90.4%)	15 (10;22)	169
EEAA	65 (89%)	31 (20;87)	7932
BAA	70 (95.9%)	30 (20;47)	558
PhAA	72 (98.6%)	275 (146;498)	43261
2-MPA	71 (97.3%)	20 (13;24)	192

LD = 3 µg/L.
[a] Before imputation.

Table 4
Go/No-Go task performance indicators (n = 72).

Indicators	Median (Q1;Q3)
Hit rate (in %)	98.4 (96.4;99.6)
Commission rate (in %)	23.1 (14.4;35.2)
Average reaction time (in ms)	393 (362;429)
Performance score	0.2 (−0.4;0.9)

rho = 0.33) but the correlations were not statistically significant (p = 0.50, p = 0.64, and p = 0.87, respectively).

3.3. Performance indicators and brain activation during the task

Children performed the Go/No-Go task with a median hit rate of 98.4%, 23.1% incorrect answers at No-Go cues, and response latency of 393 ms. The PS ranged from −9.9 to 2.1, with a median of 0.2 (Table 4).

For the "No-go *vs* Go" contrast in brain, children showed activation in regions known to be involved in the motor inhibition network: the anterior cingulate/SMA and the inferior and middle frontal regions of both hemispheres. This contrast was also associated with activated clusters in the right middle temporal region and brain structures of both hemispheres (parietal region, posterior lobe of cerebellum and caudate nucleus) (see Supplementary material, Table S.3). Similar activations were found with contrasts that took into account children's responses, *i.e.* "Successful No-Go vs successful Go" or "Failed No-Go vs successful Go" (see Supplementary material, Tables S.4 and S.5).

3.4. Prenatal urinary glycol ether metabolite levels and motor inhibition

We observed no association between commission rate and urinary levels of glycol ether metabolites (Fig. I), although there was a statistical trend for a lower commission rate for children whose mothers had high PhAA urinary levels (decrease of 6.4%, p = 0.09). Children whose mothers had moderate urinary EEAA levels had also a 0.08% faster reaction time than those whose mothers had low levels, but there was no statistically significant increase of the PS (p = 0.10). There was a statistical trend for a lower PS (decrease of 0.3 units, p = 0.06) with increasing concentrations of EAA in maternal urine. The highest levels of BAA were associated with a longer reaction time (increase of 0.08%, p = 0.04) and lower PS (decrease of 1.1 units, p = 0.02). Our results showed a linear dose-response relation between the children's PS and maternal urinary levels of BAA, when BAA concentrations were used as a continuous variable.

There were no statistically significant differences of activation for regions involved in the motor inhibition network (frontal and anterior cingular cortices and SMA) in association with motor inhibition demand for any urinary levels of glycol ether metabolites (Table 5). There was increased activation in occipital areas associated with moderate urinary levels of MAA and EAA. Children whose mothers had moderate levels of MAA exhibited higher activation in the right precuneus region and children whose mothers had moderate levels of EAA exhibited higher activation in the left cuneus region (Table 5 and Fig. II).

We found differential activities in opposite directions in brain regions related to inhibition network (frontal cortex) when children succeeded to inhibit in association with urinary levels of EEAA and PhAA but not with other glycol ether metabolites. There were decreased activities in left temporal (β = −0.96, 95% CI = [−1.23; −0.68]) and medial frontal regions (β = −1.1, 95% CI = [−1.47; −0.72]) associated with high levels of EEAA. We found increased activity in right inferior frontal region (β = 2.0, 95% CI = [1.2; 2.9]) in association with moderate levels of PhAA (Table 6 and Fig. III). These two findings were not observed when children failed to inhibit (see Supplementary material, Table S.6). Increased activations observed with motor inhibition demand in right precuneus and left cuneus (with MAA and EAA levels, respectively) were still found when distinguishing successful and failed inhibition but did not remain statistically significant after correction for multiple testing (Table 6 and Supplementary material, Table S.6).

4. Discussion

Our study shows differential performance during motor inhibition in children associated with their mothers' urinary glycol ether metabolite levels measured before 19 weeks of gestation. Higher maternal BAA urinary levels were associated with poorer performance of the child and increased reaction time. There was a linear trend of poorer performance in association with increasing levels of EAA. Moderate levels of EEAA in maternal urine were associated with a decreased reaction time, but no statistically significant difference in the PS score. There was no association between maternal urinary concentrations of metabolites and neural activity related to inhibition demand in regions involved in the motor inhibition network. We reported associations between increased child brain activation in the left cuneus and moderate levels of EAA and increased activity in the right precuneus and moderate MAA concentrations in maternal urine. We observed associations between decreased child brain activation related to successful inhibition in frontal regions, part of the inhibition network, and high levels of EEAA and between increased activity and moderate PhAA concentrations in maternal urine (Table 7).

No previous studies have used fMRI to investigate potential associations between prenatal exposure to solvents and motor inhibition function, but existing studies have evaluated associations between heavy *in-utero* exposure to a well-known solvent, namely alcohol, and brain activation during a motor inhibition task. The authors reported similar performance between exposed children and controls (n = 13/9 (Fryer et al., 2007), n = 8/17 (Kodali et al., 2017), n = 20/15 (O'Brien et al., 2013) and n = 21/21 (Ware et al., 2015)) aged 8 to 18 years, but observed differential brain activity in regions involved in motor inhibition (prefrontal, frontal, and cingular cortices). They also reported differential neural activity in regions involved in attention (parietal cortex, precuneus, cuneus, and caudate nucleus regions). Here, we used fMRI to investigate brain functioning during an inhibition task in association with glycol ether exposure in the general population. This technique provides excellent spatial resolution, giving the ability to investigate potential effects of exposure by fine brain regions of a few cubic millimeters. In addition, our study was based on a mother-child cohort (PELAGIE study), which gives the opportunity to measure glycol ether exposure before 19 weeks of gestation. The beginning of gestation is a key period for neurodevelopment and its disruption may result in short and long-term effects, such as behavioral and skill impairment (Julvez and Grandjean, 2009b; Rice and Barone, 2000). We used urinary biomarkers to estimate glycol ether exposure. Because glycol ethers are used in both occupational and household products, biomarkers enable the assessment of all sources of exposure, but without knowledge of the main route. We were also able to consider lifestyle and socioeconomic factors by restriction or adjustment. In particular, children whose mothers reported alcohol or tobacco consumption during pregnancy were not included in the study to eliminate two established risk factors of poorer neurodevelopment (Julvez and Grandjean, 2009b).

In their review, Bari and Robbins distinguished two major types of inhibitory control, cognitive and behavioral (Bari and Robbins, 2013). Here, the Go/No-Go task involves behavioral response inhibition with a motor component. This function appears in childhood and continues to mature into adolescence (Booth et al., 2003; Luna and Sweeney, 2006). All children understood and performed the Go/No-Go task well. Thus, response inhibition appeared to be well-established in our population of 10- to 12-year-old children. Performances in our study are similar to that reported during a similar Go/No-Go task with 8- to 13-year-old

(caption on next page)

Fig. I. Associations between glycol ether metabolite levels in maternal urine during pregnancy and performance indicators for the Go/No-Go task of their 10- to 12-year-old children (PELAGIE cohort, 2002–2017) (n = 72).
Abbreviations: BAA, 2-butoxyacetic acid; EAA, ethoxyacetic acid; EEAA, ethoxyethoxyacetic acid; MAA, methoxyacetic acid; 2-MPA, 2-methoxyproprionic acid; PhAA, phenoxyacetic acid; ref.: reference.
β (95% IC): estimated coefficient [95% confidence interval].
Adjusted for maternal educational level (< *vs* ≥3 years of post-secondary school attendance) and child's breastfeeding (≤ *vs* > 3 months).
*p ≤ 0.05, †: 0.05 < p ≤ 0.10.
a: Log-scale.
b: p-Value from linear trend-test.

children (average latency of 393 ms *vs* 407 ms, 98% *vs* 97.2% hit rate, and 23% *vs* 22% for commission errors) (Suskauer et al., 2008). Three main regions (the anterior cingulate, SMA, and inferior frontal gyri of both hemispheres) are involved in motor inhibition (Allman et al., 2006; Aron, 2007; Mostofsky et al., 2003). As expected, we observed cerebral activation in this network during inhibitory control demand ("No-Go *vs* Go" contrast). We also observed additional activation in other brain structures of both hemispheres (parietal, including the right cuneus and precuneus regions, temporal gyri, caudate nucleus, and posterior lobe of the cerebellum), also in 8- to 13-year-old children (Suskauer et al., 2008). These regions do not appear to be part of the motor inhibition network and may be related to attention and decision making processes (Behrmann et al., 2004; Grahn et al., 2008; Stoodley and Schmahmann, 2009).

Table 5
Associations between glycol ether metabolite levels in maternal urine during pregnancy and brain activation intensity during the Go/No-Go task of their 10- to 12-year-old children (PELAGIE cohort, 2002–2017) (n = 71).

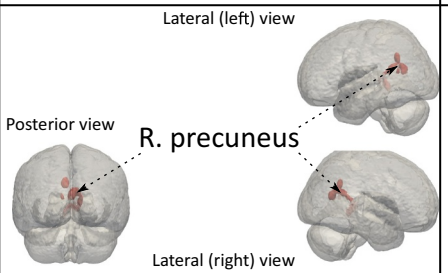
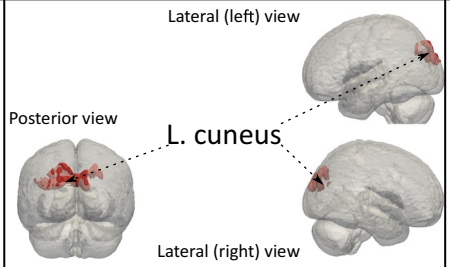
Metabolite concentrations (in µg/L)	N	Frontal or cingular cortices		Brain regions outside frontal and cingular cortices		
		β (95% CI)	ext.	β (95% CI)	Region	ext.
MAA						
Low: < 52.4	26	Ref.		Ref.		
Moderate: [52.4–82.9]	23			1.3 (0.6, 1.9)*	R. precuneus	480
High: ≥82.9	22			0.6 (−0.01; 1.3)[a]	R. precuneus	480
EAA						
Low: < 9.7	29	Ref.		Ref.		
Moderate: [9.7–22]	22			1.7 (1.0, 2.3)*	L. cuneus	898
High: ≥22	20			0.06 (0; 1.1)[a]	L. cuneus	898
EEAA						
Low: < 19.8	30	Ref.		Ref.		
Moderate: [19.8–86.5]	21					
High: ≥86.5	20					
BAA						
Low: < 19.7	27	Ref.		Ref.		
Moderate: [19.7–47.1]	23					
High: ≥47.1	21					
PhAA						
Low: < 146.4	25	Ref.		Ref.		
Moderate: [146.4–498.1]	24					
High: ≥498.1	22					
2-MPA						
Low: < 13.2	26	Ref.		Ref.		
Moderate: [13.2–24.1]	21					
High: ≥24.1	24					

Abbreviations: BAA, 2-butoxyacetic acid; EAA, ethoxyacetic acid; EEAA, ethoxyethoxyacetic acid; ext.: extent (in voxels); MAA, methoxyacetic acid; 2-MPA, 2-methoxyproprionic acid; PhAA, phenoxyacetic acid; ref.: reference.
β (95% IC): estimated coefficient [95% confidence interval]. Empty cell: no statistically significant difference reported in this region.
Adjusted for maternal educational level (< *vs* ≥3 years of post-secondary school attendance) and child's breastfeeding (≤ *vs* > 3 months).
* FWER correction at cluster level, p < 0.05.
[a] Coefficient and confidence interval estimated for the statistically significant clusters determined for moderate levels of exposure.

One of our performance indicators to measure impulsivity was the proportion of commission errors, for which we observed no significant association between glycol ether exposures. We cannot eliminate the possibility that all children of our population, even those with problems concentrating, were able to focus for 10 min and that the task was too short to discriminate between the children. We observed that children making few commission errors tended to be slower. Thus, children with inhibition problems may also develop adaptive strategies, such as slowing their response speed.

We observed an association between increased maternal urinary levels of BAA and poorer PS, as well as increased reaction time, whereas the commission rate remained unchanged. Concentrations of BAA in maternal urine were not associated with differential neural activity during the task. If there is an association between BAA urinary levels and brain functioning, it is possible that our methodological choices (MRI sequence, sample size) did not allow to measure it. Urinary metabolite levels of BAA are weakly correlated with the concentration of other metabolites, although its parent compounds are used in the same products as the other glycol ethers measured in this study. BAA is the main metabolite of ethylene glycol butyl monobutyl ether (EGBE) and diethylene glycol butyl monobutyl ether (DEGBE), two glycol ethers widely used in Europe (2nd and 4th in commercialized volumes of glycol ethers during the study period). They are found in 20% of industrial paints and varnishes (DEGBE), 10% of biocides, and in cleaning products and cosmetic products (up to 0.2% in hair dyes). EGBE is well-known for its hemolytic properties, but not for neurotoxicity (AFSSET, 2008; INRS, 2011).

We observed weaker associations between maternal urinary levels of EAA or EEAA and motor inhibition indicators. There was a slight decrease in reaction time with moderate EEAA levels and decreased cerebral activities in inhibition network (in frontal cortex) when children succeeded to inhibit their answer with high levels of EEAA. There was a trend towards poorer performance with higher levels of EAA. There were no statistically significant associations between BOLD responses in brain regions involved in the motor inhibition network and urinary levels of EAA, but rather an association between urinary levels of these glycol ethers and an increased BOLD response in the left cuneus, a region involved in attention and reported in similar Go/No-Go studies (Cui et al., 2009; Suskauer et al., 2008). The cuneus is located in the occipital part of the brain and is involved in visual preprocessing (Rosen et al., 2018). The difference of brain activity in this region may be consistent with visual alterations reported by Till et al. in association with prenatal occupational exposure to solvents. EAA is a metabolite of EGEE, EGDEE, DEGEE, DEGDEE, and TEGEE. EAA levels are correlated with those of EEAA, which is also a metabolite of DEGEE and TEGEE. At the time of the study, these glycol ethers were found in paint, ink, cleaning products, and biocides (AFSSET, 2008; INRS, 2011). Some ethoxyethanol (EGEE) derivatives are recognized for their reproductive and developmental toxicity, but there are few studies on their neurotoxicity.

	1st tercile	2nd tercile (vs 1st)	3rd tercile (vs 1st)
MAA	Reference		*No statistically significant difference*
EAA	Reference		*No statistically significant difference*
EEAA	Reference	*No statistically significant difference*	*No statistically significant difference*
BAA	Reference	*No statistically significant difference*	*No statistically significant difference*
PhAA	Reference	*No statistically significant difference*	*No statistically significant difference*
2-MPA	Reference	*No statistically significant difference*	*No statistically significant difference*

(caption on next page)

Fig. II. Summary of associations (estimated coefficients) between urinary glycol ether metabolite levels during pregnancy and brain activation during motor inhibition demand (n = 71)*.
Abbreviations: BAA, 2-butoxyacetic acid; EAA, ethoxyacetic acid; EEAA, ethoxyethoxyacetic acid; L.: left; MAA, methoxyacetic acid; 2-MPA, 2-methoxyproprionic acid; PhAA, phenoxyacetic acid; R.: right.
*FWER corrected-p < 0.05 with a one-sided uncorrected-p = 0.01 threshold.

There were an increased BOLD response in regions involved in shifting attention (precuneus) in association with moderate urinary levels of MAA (Cavanna and Trimble, 2006; Suskauer et al., 2008). MAA comes from the metabolism of EGME, EGDME, DEGME, DEGDME, TEGME, and TEGDME. Products containing > 0.5% EGME were prohibited in France before establishment of the PELAGIE cohort. EGDME, DEGDME, and TEGDME were banned in 2005 but DEGME and TEGME are still authorized and used in cleaning agents (AFSSET, 2008; INRS, 2011). Some methoxyethanol (EGME) derivatives are recognized for their reproductive and developmental toxicity, but there are few studies on their neurotoxicity.

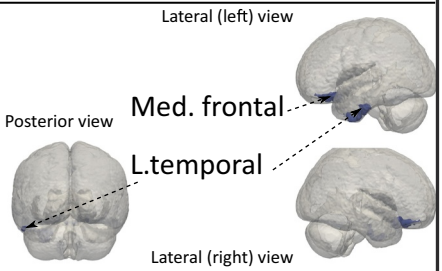
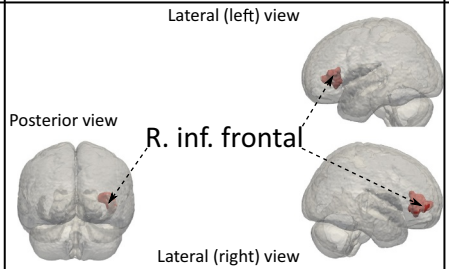
We did not report any association between performance and urinary levels of PhAA, only a slight decrease of commission errors with increasing metabolite levels. There was also an increased activity in relation with moderate level of PhAA in inhibition network (frontal cortex) when children succeeded to inhibit their answer. EGPhE (parent compound of PhAA) is used as preservatives up to 1% in half cosmetic products in France, and some biocides and pharmaceutical products (AFSSET, 2008).

The possibly causal interpretation of our results is very limited by the lack of studies on the developmental neurotoxicity of glycol ethers, even mechanistic or *in-vivo* studies. In a previous epidemiological study from the PELAGIE cohort, Béranger et al. (2017) reported associations between maternal urinary concentrations of PhAA and verbal comprehension scores and EAA levels and visuospatial performance (n = 204), brain functions which could be controlled by cognitive inhibition. Here, we only observe an association between moderate PhAA metabolite levels and cerebral activity in frontal cortex and decreased commission rates with increasing levels of urinary biomarker. Outside inhibition network we observed increased activity in the left cuneus and a trend towards poorer performance with EAA metabolite concentrations. In contrast, we found associations between BAA levels in maternal urine and poorer inhibition performance and differential brain activity in relation with EEAA levels, although these glycol ether metabolites do not appear to be associated with poorer verbal comprehension or visuospatial abilities at six years of age (Béranger et al., 2017). An animal study has suggested behavioral alterations (learning and neuromuscular abilities) and variations of the cerebral concentrations of neurotransmitters (acetylcholine, norepinephrine, dopamine, and serotonin) in rats prenatally exposed to EAA and MAA through their parent

Table 6
Associations between glycol ether metabolite levels in maternal urines during pregnancy and activation intensity related to successful inhibition during the Go/No-Go task of their 10–12 years old children (PELAGIE cohort. 2002–2017) (FWER correction at cluster level. p < 0.05) (n = 70).

Metabolite concentrations (in µg/L)	N	Frontal or cingular cortices		Brain regions outside frontal and cingular cortices		
		β (95% CI)	ext.	β (95% CI)	Region	ext.
MAA						
Low: < 52.4	25	Ref.		Ref.		
Moderate: [52.4–82.9]	23			1.0 (0.3; 1.7)[a]	R. precuneus	480
High: ≥82.9	22					
EAA						
Low: < 9.7	29	Ref.		Ref.		
Moderate: [9.7–22]	21			0.7 (0.2; 1.4)[a]	L. cuneus	898
High: ≥22	20					
EEAA						
Low: < 19.8	30	Ref.		Ref.		
Moderate: [19.8–86.5]	20					
High: ≥86.5	20	−1.1 (−1.47; −0.72)	510	−0.96 (−1.23; −0.68)	Left temporal	499
BAA						
Low: < 19.7	27	Ref.		Ref.		
Moderate: [19.7–47.1]	23					
High: ≥47.1	20					
PhAA						
Low: < 146.4	25	Ref.		Ref.		
Moderate: [146.4–498.1]	24	2.0 (1.2; 2.9)	524			
High: ≥498.1	21					
2-MPA						
Low: < 13.2	25	Ref.		Ref.		
Moderate: [13.2–24.1]	21					
High: ≥24.1	24					

Adjusted for maternal educational level (< *vs* ≥3 years of post-secondary school attendance) and child's breastfeeding (≤ *vs* > 3 months). β [95% IC]: estimated coefficient [95% confidence interval]. ext.: cluster extent (in voxels). ref.: reference. Empty cell: no statistically significant difference reported in this region.
BAA. 2-butoxyacetic acid; EAA. ethoxyacetic acid; EEAA. ethoxyethoxyacetic acid; MAA. methoxyacetic acid; 2-MPA. 2-methoxyproprionic acid; PhAA. phenoxyacetic acid.
[a] Coefficient and confidence interval estimated for the statistically significant clusters determined for moderate levels of exposure in Table 5.

	1st tercile	2nd tercile (vs 1st)	3rd tercile (vs 1st)
MAA	Reference	*No statistically significant difference*	*No statistically significant difference*
EAA	Reference	*No statistically significant difference*	*No statistically significant difference*
EEAA	Reference	*No statistically significant difference*	
BAA	Reference	*No statistically significant difference*	*No statistically significant difference*
PhAA	Reference		*No statistically significant difference*
2-MPA	Reference	*No statistically significant difference*	*No statistically significant difference*

(caption on next page)

Fig. III. Summary of associations (estimated coefficients) between urinary glycol ether metabolite levels during pregnancy and brain activation during successful inhibition (n = 70)*.
Abbreviations: BAA, 2-butoxyacetic acid; EAA, ethoxyacetic acid; EEAA, ethoxyethoxyacetic acid; L.: left; MAA, methoxyacetic acid; 2-MPA, 2-methoxyproprionic acid; PhAA, phenoxyacetic acid; R.: right.
*FWER corrected-p < 0.05 with a one-sided uncorrected-p = 0.01 threshold.

Table 7
Summary of the statistically significant associations between glycol ether metabolite levels in maternal urines during pregnancy and motor inhibition of their 10–12 years old children (PELAGIE cohort. 2002–2017).

Metabolite concentrations (in µg/L)	Performances	Brain activity	
		Inhibition network	Others brain regions
MAA			
Low: < 52.4	Ref.	Ref.	
Moderate: [52.4–82.9]			↗ R precuneus
High: ≥ 82.9			
EAA			
Low: < 9.7	Ref.	Ref.	
Moderate: [9.7–22]			↗ L cuneus
High: ≥ 22			
EEAA			
Low: < 19.8	Ref.	Ref.	
Moderate: [19.8–86.5]	↘ reaction time		
High: ≥ 86.5		↘ med frontal	↘ L temporal
BAA			
Low: < 19.7	Ref.	Ref.	
Moderate: [19.7–47.1]	↗ reaction time, ↘ PS score		
High: ≥ 47.1	↘ PS score		
PhAA			
Low: < 146.4	Ref.	Ref.	
Moderate: [146.4–498.1]		↗ R inferior frontal	
High: ≥ 498.1			
2-MPA			
Low: < 13.2	Ref.	Ref.	
Moderate: [13.2–24.1]			
High: ≥ 24.1			

(respectively EGEE and EGME), at doses that showed no effect on their mothers and equivalent to the US permissible limit (for EGME) or half the limit (for EGEE) (Nelson et al., 1984; Nelson and Brightwell, 1984). There are no existing studies on the possible developmental neurotoxicity of EGBE.

This study had some limitations. We cannot exclude a selection bias, although we did not find any difference in the characteristics of our study population and non-participants, limiting the risk that a confounder may predict participation. Moreover, we investigated subtle behavioral effects, which would be less sensitive to attrition bias than apparent clinical effects (Greene et al., 2011). BOLD imaging measures the ratio of deoxyhemoglobin to oxyhemoglobin to indirectly evaluate neural activity. This indicator is commonly used, but may vary depending on individual characteristics (blood flow, iron deficiency, *etc.*) (Ogawa et al., 1990). Moreover, the performance of inhibition is not determined solely by intensity of the hemodynamic response and could be explained by brain structure, connectivity, *etc.* Also, our exposure assessment is based on only one (first) morning void urinary sample collected before 19 weeks of gestation, and glycol ether metabolites have a short half-life, from several hours to several days (AFSSET, 2008). However, the high detection rate of glycol ether metabolites suggests that exposure was widespread and repeated. In addition, a previous study in the PELAGIE cohort found associations between the use of products containing solvents during early pregnancy and the urinary levels of four glycol ether metabolites (EAA, EEAA, BAA, and PhAA) (Garlantézec et al., 2012). Another cohort study highlighted the lack of information provided to French pregnant women concerning the prevention of exposure to reprotoxicants, including housekeeping products, and a French study suggested that cosmetics are generally used daily at home, including during pregnancy (Chabert et al., 2016; Ficheux et al., 2015). Thus, our results likely reflect regular individual exposure during the first trimester of pregnancy, a critical period for brain development. However, repeated samples would have been needed to ensure this hypothesis. Similarly, it is likely that our measurement did not capture intra-individual variability, over time, due to the rapid metabolism of glycol ethers. This produces non-differential measurement errors in the exposition estimation, which could have been limited by using repeated samples. Our study does not explore the effects of glycol ether exposure during late pregnancy or childhood, which could occur *via* various sources and exposure pathways, although human neurodevelopment is known to continue during childhood, until adolescence for maturation of the prefrontal cortex (Rice and Barone, 2000). Although we considered significant confounders and based our study on a homogeneous population, we cannot guarantee that our results were not affected by residual confounding. Thus, given that prenatal exposure to products containing solvents at work has been associated with problems related to the function of inhibition, we cannot exclude that our results may be explained by other solvents or chemicals present in these products (Laslo-Baker et al., 2004; Pelé et al., 2013; Till et al., 2001a,b). Then, we do not correct analyses for multiple comparisons with glycol ethers to protect against false negative results but we cannot rule out the possibility that some of them are due to chance. Finally, we cannot rule out the hypothesis of a lack of statistical power, especially for our whole-brain strategy.

5. Conclusion

Human exposure to glycol ethers is ubiquitous in our daily lives and might be of particular concern for pregnant women and developing fetuses. According to previous observational epidemiological studies, some glycol ethers have been suspected as neurodevelopmental toxicants, especially for learning abilities and behavioral development, while too few mechanistic studies supporting this possible effect exist. The present study provides new findings suggesting differential activations in the brain motor inhibition network in association with prenatal glycol ethers exposure. However, some results about specific glycol ether metabolites were unexpected regarding existing literature. Therefore, replication of these findings and deeper investigation on possible neural development changes are strongly required before drawing any conclusions or public health recommendations.

Declaration of competing interest

None.

Acknowledgements

We are grateful to the gynecologists, obstetricians, ultra-sonographers, midwives, pediatricians, and families who participated in the study. We particularly thank Catherine Ducassoux and the MR technicians for neuropsychological and MRI testing.

This study is supported by the Fondation de France (FdF) and Rennes University Hospital. The PELAGIE cohort is funded by Inserm, the French Ministries of Health, Labour, and Ecology, InVS, ANR, and

ANSES. MRI data acquisition was performed at the Neurinfo MRI research facility of the University of Rennes I. Neurinfo is funded by the European Union (FEDER), the French State, the Brittany Council, Rennes Metropole, INRIA, Inserm, and the University Hospital of Rennes.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105163.

References

AFSSET, 2008. Synthèse des connaissances sur les expositions de la population générale et professionnelle en France. [WWW Document]. URL. https://www.anses.fr/fr/system/files/CHIM2003et0016Ra-3.pdf, Accessed date: 19 September 2018.

Allman, J.M., Hakeem, A., Erwin, J.M., Nimchinsky, E., Hof, P., 2006. The anterior cingulate cortex. Ann. N. Y. Acad. Sci. 935, 107–117. https://doi.org/10.1111/j.1749-6632.2001.tb03476.x.

Aron, A.R., 2007. The neural basis of inhibition in cognitive control. Neuroscientist 13, 214–228. https://doi.org/10.1177/1073858407299288.

Bari, A., Robbins, T.W., 2013. Inhibition and impulsivity: behavioral and neural basis of response control. Prog. Neurobiol. 108, 44–79. https://doi.org/10.1016/j.pneurobio.2013.06.005.

Behrmann, M., Geng, J.J., Shomstein, S., 2004. Parietal cortex and attention. Curr. Opin. Neurobiol. 14, 212–217. https://doi.org/10.1016/j.conb.2004.03.012.

Béranger, R., Garlantézec, R., Le Maner-Idrissi, G., Lacroix, A., Rouget, F., Trowbridge, J., Warembourg, C., Monfort, C., Le Gléau, F., Jourdin, M., Multigner, L., Cordier, S., Chevrier, C., 2017. Prenatal exposure to glycol ethers and neurocognitive abilities in 6-year-old children: the PELAGIE cohort study. Environ. Health Perspect. 125, 684–690. https://doi.org/10.1289/EHP39.

Booth, J.R., Burman, D.D., Meyer, J.R., Lei, Z., Trommer, B.L., Davenport, N.D., Li, W., Parrish, T.B., Gitelman, D.R., Mesulam, M.M., 2003. Neural development of selective attention and response inhibition. NeuroImage 20, 737–751. https://doi.org/10.1016/S1053-8119(03)00404-X.

Cartier, C., Warembourg, C., Le Maner-Idrissi, G., Lacroix, A., Rouget, F., Monfort, C., Limon, G., Durand, G., Saint-Amour, D., Cordier, S., Chevrier, C., 2016. Organophosphate insecticide metabolites in prenatal and childhood urine samples and intelligence scores at 6 years of age: results from the mother–child PELAGIE cohort (France). Environ. Health Perspect. 124, 674–680. https://doi.org/10.1289/ehp.1409472.

Cavanna, A.E., Trimble, M.R., 2006. The precuneus: a review of its functional anatomy and behavioural correlates. Brain 129, 564–583. https://doi.org/10.1093/brain/awl004.

Chabert, M.-C., Perrin, J., Berbis, J., Bretelle, F., Adnot, S., Courbiere, B., 2016. Lack of information received by a French female cohort regarding prevention against exposure to reprotoxic agents during pregnancy. Eur. J. Obstet. Gynecol. Reprod. Biol. 205, 15–20. https://doi.org/10.1016/j.ejogrb.2016.07.504.

Collignon, O., Girard, S., Gosselin, F., Saint-Amour, D., Lepore, F., Lassonde, M., 2010. Women process multisensory emotion expressions more efficiently than men. Neuropsychologia 48, 220–225. https://doi.org/10.1016/j.neuropsychologia.2009.09.007.

Cui, X., Stetson, C., Montague, P.R., Eagleman, D.M., 2009. Ready…go: amplitude of the fMRI signal encodes expectation of cue arrival time. PLoS Biol. 7, e1000167. https://doi.org/10.1371/journal.pbio.1000167.

Diamond, A., Barnett, W.S., Thomas, J., Munro, S., 2007. Preschool program improves cognitive control. Science 318, 1387–1388. https://doi.org/10.1126/science.1151148.

Eskenazi, B., Gaylord, L., Bracken, M.B., Brown, D., 1988. In utero exposure to organic solvents and human neurodevelopment. Dev. Med. Child Neurol. 30, 492–501. https://doi.org/10.1111/j.1469-8749.1988.tb04776.x.

Ficheux, A.S., Wesolek, N., Chevillotte, G., Roudot, A.C., 2015. Consumption of cosmetic products by the French population. First part: frequency data. Food Chem. Toxicol. 78, 159–169. https://doi.org/10.1016/j.fct.2015.01.016.

Fromme, H., Nitschke, L., Boehmer, S., Kiranoglu, M., Göen, T., 2013. Exposure of German residents to ethylene and propylene glycol ethers in general and after cleaning scenarios. Chemosphere 90, 2714–2721. https://doi.org/10.1016/j.chemosphere.2012.11.051.

Fryer, S.L., Tapert, S.F., Mattson, S.N., Paulus, M.P., Spadoni, A.D., Riley, E.P., 2007. Prenatal alcohol exposure affects frontal–striatal bold response during inhibitory control. Alcohol. Clin. Exp. Res. 31, 1415–1424. https://doi.org/10.1111/j.1530-0277.2007.00443.x.

Garlantézec, R., Monfort, C., Rouget, F., Cordier, S., 2009. Maternal occupational exposure to solvents and congenital malformations: a prospective study in the general population. Occup. Environ. Med. 66, 456–463. https://doi.org/10.1136/oem.2008.041772.

Garlantézec, R., Multigner, L., Labat, L., Bonvallot, N., Pulkkinen, J., Dananché, B., Monfort, C., Rouget, F., Cordier, S., 2012. Urinary biomarkers of exposure to glycol ethers and chlorinated solvents during pregnancy: determinants of exposure and comparison with indirect methods of exposure assessment. Occup. Environ. Med. 69, 62–70. https://doi.org/10.1136/oem.2010.062315.

Garlantézec, R., Warembourg, C., Monfort, C., Labat, L., Pulkkinen, J., Bonvallot, N., Multigner, L., Chevrier, C., Cordier, S., 2013. Urinary glycol ether metabolites in women and time to pregnancy: the PELAGIE cohort. Environ. Health Perspect. 121, 1167–1173. https://doi.org/10.1289/ehp.1206103.

Grahn, J.A., Parkinson, J.A., Owen, A.M., 2008. The cognitive functions of the caudate nucleus. Prog. Neurobiol. 86, 141–155. https://doi.org/10.1016/j.pneurobio.2008.09.004.

Grandjean, P., Landrigan, P., 2006. Developmental neurotoxicity of industrial chemicals. Lancet 368, 2167–2178. https://doi.org/10.1016/S0140-6736(06)69665-7.

Greene, N., Greenland, S., Olsen, J., Nohr, E.A., 2011. Estimating bias from loss to follow-up in the Danish National Birth Cohort. Epidimiology 22, 815–822. https://doi.org/10.1097/EDE.0b013e31822939fd.

INRS, 2011. Les éthers de glycol [WWW Document]. URL. http://www.inrs.fr/dms/inrs/CataloguePapier/ED/TI-ED-4222/ed4222.pdf, Accessed date: 19 September 2018.

Jin, Y., Hein, M.J., Deddens, J.A., Hines, C.J., 2011. Analysis of lognormally distributed exposure data with repeated measures and values below the limit of detection using SAS. Ann. Occup. Hyg. 55, 97–112. https://doi.org/10.1093/annhyg/meq061.

Julvez, J., Grandjean, P., 2009a. Neurodevelopmental toxicity risks due to occupational exposure to industrial chemicals during pregnancy. Ind. Health 47, 459–468.

Julvez, J., Grandjean, P., 2009b. Neurodevelopmental toxicity risks due to occupational exposure to industrial chemicals during pregnancy. Ind. Health 47, 459–468. https://doi.org/10.2486/indhealth.47.459.

Kodali, V.N., Jacobson, J.L., Lindinger, N.M., Dodge, N.C., Molteno, C.D., Meintjes, E.M., Jacobson, S.W., 2017. Differential recruitment of brain regions during response inhibition in children prenatally exposed to alcohol. Alcohol. Clin. Exp. Res. 41, 334–344. https://doi.org/10.1111/acer.13307.

Laslo-Baker, D., Barrera, M., Knittel-Keren, D., Kozer, E., Wolpin, J., Khattak, S., Hackman, R., Rovet, J., Koren, G., 2004. Child neurodevelopmental outcome and maternal occupational exposure to solvents. Arch. Pediatr. Adolesc. Med. 158, 956–961. https://doi.org/10.1001/archpedi.158.10.956.

Luna, B., Sweeney, J.A., 2006. The emergence of collaborative brain function: fMRI studies of the development of response inhibition. Ann. N. Y. Acad. Sci. 1021, 296–309. https://doi.org/10.1196/annals.1308.035.

Moffitt, T.E., Arseneault, L., Belsky, D., Dickson, N., Hancox, R.J., Harrington, H., Houts, R., Poulton, R., Roberts, B.W., Ross, S., Sears, M.R., Thomson, W.M., Caspi, A., 2011. A gradient of childhood self-control predicts health, wealth, and public safety. Proc. Natl. Acad. Sci. 108, 2693–2698. https://doi.org/10.1073/pnas.1010076108.

Mostofsky, S.H., Schafer, J.G., Abrams, M.T., Goldberg, M.C., Flower, A.A., Boyce, A., Courtney, S.M., Calhoun, V.D., Kraut, M.A., Denckla, M.B., et al., 2003. fMRI evidence that the neural basis of response inhibition is task-dependent. Cogn. Brain Res. 17, 419–430.

Nelson, B.K., Brightwell, W.S., 1984. Behavioral teratology of ethylene glycol monomethyl and monoethyl ethers. Environ. Health Perspect. 57, 43–46.

Nelson, B.K., Brightwell, W.S., Setzer, J.V., O'Donohue, T.L., 1984. Reproductive toxicity of the industrial solvent 2-ethoxyethanol in rats and interactive effects of ethanol. Environ. Health Perspect. 57, 255–259.

Nichols, T., Hayasaka, S., 2003. Controlling the familywise error rate in functional neuroimaging: a comparative review. Stat. Methods Med. Res. 12, 419–446. https://doi.org/10.1191/0962280203sm341ra.

O'Brien, J.W., Norman, A.L., Fryer, S.L., Tapert, S.F., Paulus, M.P., Jones, K.L., Riley, E.P., Mattson, S.N., 2013. Effect of predictive cuing on response inhibition in children with heavy prenatal alcohol exposure. Alcohol. Clin. Exp. Res. 37, 644–654. https://doi.org/10.1111/acer.12017.

Ogawa, S., Lee, T.M., Kay, A.R., Tank, D.W., 1990. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc. Natl. Acad. Sci. U. S. A. 87, 9868–9872.

Pelé, F., Muckle, G., Costet, N., Garlantézec, R., Monfort, C., Multigner, L., Rouget, F., Cordier, S., 2013. Occupational solvent exposure during pregnancy and child behaviour at age 2. Occup. Environ. Med. 70, 114–119.

Réveillon, M., Urben, S., Barisnikov, K., Borradori Tolsa, C., Hüppi, P.S., Lazeyras, F., 2013. Functional neuroimaging study of performances on a Go/No-go task in 6- to 7-year-old preterm children: impact of intrauterine growth restriction. NeuroImage Clin. 3, 429–437. https://doi.org/10.1016/j.nicl.2013.10.007.

Rice, D., Barone, S., 2000. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ. Health Perspect. 108, 511–533. https://doi.org/10.1289/ehp.00108s3511.

Rosen, M.L., Sheridan, M.A., Sambrook, K.A., Peverill, M.R., Meltzoff, A.N., McLaughlin, K.A., 2018. The role of visual association cortex in associative memory formation across development. J. Cogn. Neurosci. 30, 365–380. https://doi.org/10.1162/jocn_a_01202.

Stoodley, C.J., Schmahmann, J.D., 2009. Functional topography in the human cerebellum: a meta-analysis of neuroimaging studies. NeuroImage 44, 489–501. https://doi.org/10.1016/j.neuroimage.2008.08.039.

Suskauer, S.J., Simmonds, D.J., Fotedar, S., Blankner, J.G., Pekar, J.J., Denckla, M.B., Mostofsky, S.H., 2008. Functional magnetic resonance imaging evidence for abnormalities in response selection in attention deficit hyperactivity disorder: differences in activation associated with response inhibition but not habitual motor response. J. Cogn. Neurosci. 20, 478–493. https://doi.org/10.1162/jocn.2008.20032.

Till, C., Koren, G., Rovet, J.F., 2001a. Prenatal exposure to organic solvents and child neurobehavioral performance. In: Neurotoxicol. Teratol., Twenty-Fifth Annual Meeting of the Neurobehavioral Teratology Society. vol 23. pp. 235–245. https://doi.org/10.1016/S0892-0362(01)00141-6.

Till, C., Westall, C.A., Rovet, J.F., Koren, G., 2001b. Effects of maternal occupational exposure to organic solvents on offspring visual functioning: a prospective controlled study. Teratology 64, 134–141. https://doi.org/10.1002/tera.1056.

Till, C., Westall, C.A., Koren, G., Nulman, I., Rovet, J.F., 2005. Vision abnormalities in young children exposed prenatally to organic solvents. Neurotoxicology 26, 599–613.

https://doi.org/10.1016/j.neuro.2005.05.011.
Ware, A.L., Infante, M.A., O'Brien, J.W., Tapert, S.F., Jones, K.L., Riley, E.P., Mattson, S.N., 2015. An fMRI study of behavioral response inhibition in adolescents with and without histories of heavy prenatal alcohol exposure. Behav. Brain Res. 278, 137–146. https://doi.org/10.1016/j.bbr.2014.09.037.
Willcutt, E.G., Doyle, A.E., Nigg, J.T., Faraone, S.V., Pennington, B.F., 2005. Validity of the executive function theory of attention-deficit/hyperactivity disorder: a meta-analytic review. Biol. Psychiatry 57, 1336–1346. https://doi.org/10.1016/j.biopsych.2005.02.006.

ORGANOPHOSPHATE AND MOTOR INHIBITION

Status: in preparation

Binter AC, Bannier E, Saint-Amour D, Simon G, Barillot C, Monfort C, Cordier S, Pelé F*, Chevrier C*. Exposure of pregnant women to organophosphate insecticides and motor inhibition at the age of 10 to 12 years evaluated by fMRI (PELAGIE mother-child cohort).

3.1. Résumé

Contexte : Les insecticides organophosphorés (OP) sont très utilisés, notamment en agriculture. Plusieurs études épidémiologiques suggèrent une potentielle toxicité neurodéveloppementale chez l'enfant après exposition à des niveaux faibles, correspondant à ceux de la population générale, mais les mécanismes d'action restent peu compris.

Objectifs : Nous cherchons à investiguer les effets de l'exposition prénatale aux OP sur le contrôle inhibiteur d'enfants âgés de 10 à 12 ans évalué par une tâche d'inhibition motrice pendant une séquence d'IRM fonctionnelle.

Méthodes : Quatre-vingt quinze enfants de la cohorte PELAGIE (Bretagne-France, à partir de 2002) ont réalisé une tâche de Go/No-Go pendant une séquence d'IRM fonctionnelle avec la consigne suivante : appuyer le plus vite possible sur un bouton lorsqu'un smiley vert apparaissait, mais ne pas répondre aux smileys rouges (300 smileys successifs pendant 10 minutes). Les performances à la tâche ont été évaluées par le temps de réaction moyen, le taux de commission et un score composite de performance. Les activations cérébrales sur l'ensemble du cerveau ont été estimées en modélisant la réponse hémodynamique liée à la consigne d'inhibition et la réussite à supprimer le geste. L'exposition aux OP était évaluée en mesurant les concentrations de six métabolites (dialkylphosphate, DAP) dans les urines maternelles en début de grossesse (avant 19 semaines de gestation). Les concentrations ont été sommées pour obtenir les niveaux totaux de diethylphosphate (DE), dimethyphosphate (DM) et l'ensemble des métabolites non-spécifiques (DAP). Les niveaux ont été standardisés pour homogénéiser les conditions d'échantillonnage et catégorisés en 3 classes d'exposition : faible (référence), modérée et élevée. Les facteurs de confusion potentiels ont été pris en compte par restriction ou ajustement.

Résultats : Les niveaux modérés de DAP étaient associés avec une diminution du taux de commission ($p = 0.04$), sans que le score de performance augmente. On observait une tendance à la diminution du score de performance avec des niveaux modérés de DE ($p = 0.06$). L'augmentation des niveaux urinaires de DM et DE était associée avec une diminution des activités cérébrales dans les régions frontales inférieures gauches et supérieures (bilatéralement) pendant l'inhibition réussie. Il n'y

avait pas de différence d'activations pendant la demande d'inhibition.
Discussion : Nous n'avons pas trouvé d'altérations majeures des scores neuropsychologiques mais nos résultats suggèrent des diminutions de l'activité cérébrale dans des régions liées à l'inhibition après exposition prénatale aux OP. Ces résultats restent à confirmer par des études avec d'autres populations.

3.2. Prenatal exposure to OP and motor inhibition

The article resulting from the study is inserted on the next pages.

Exposure of pregnant women to organophosphate insecticides and child motor inhibition at the age of 10 to 12 years evaluated by fMRI (PELAGIE mother-child cohort, France)

Binter A.C [1], Bannier E [2], Saint-Amour D [3], Simon G [4], Barillot C [5], Monfort C [1], Cordier S [1], Pelé F [1,6*], Chevrier C [1*]

[1] Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR_S 1085, F-35000 Rennes, France

[2] Univ Rennes, CHU Rennes, CNRS, Inria, Inserm, IRISA UMR 6074, Empenn - ERL U 1228, F-35000 Rennes, France

[3] Department of Psychology, Université du Québec à Montréal, Montréal, Canada

[4] ISTS EA 7466, University of Caen Normandie, Caen, France

[5] Univ Rennes, CNRS, Inria, Inserm, IRISA UMR 6074, Empenn - ERL U 1228, F-35000 Rennes, France

[6] Univ Rennes, Inserm, CIC 1414, Rennes, France

[5] Univ Rennes, Inserm, CIC 1414, Rennes, France

* Equal contributors

Correspondence address: Inserm UMR 1085 Irset, 9 Avenue du Pr. Léon Bernard, 35000 Rennes, France, anne-claire.binter@inserm.fr

Acknowledgements: We are grateful to the gynecologists, obstetricians, ultra-sonographers, midwives, pediatricians, and families who participated in the study. We particularly thank Catherine Ducassoux and the MR technicians for neuropsychological and MRI testing.

This study is supported by the Fondation de France (FdF) and Rennes University Hospital. The PELAGIE cohort is funded by Inserm, the French Ministries of Health, Labor, and Ecology, InVS, ANR, and ANSES. MRI data acquisition was performed at the Neurinfo MRI research facility of the University of Rennes I. Neurinfo is funded by the European Union (FEDER), the French State, the Brittany Council, Rennes Metropole, Inria, Inserm, and the University Hospital of Rennes.

Conflict of interest: The authors declare they have no actual or potential competing financial interests.

Significance statement: Organophosphate pesticides (OP) are widely used for agricultural purposes. Epidemiological studies suggest neurotoxicity in children after exposure at low levels but possible mechanism is still unclear. We investigated the effects of prenatal exposure to OPs on inhibitory control of 10 to 12 year-old-children assessed by a motor inhibition task during functional magnetic

resonance imaging (fMRI). We observed decreased brain activity in regions related to inhibition after prenatal exposure to OPs, in the absence of major alteration of neuropsychological scores. These results suggest that OPs may be associated with altered pattern of brain activity and need to be confirmed by additional studies.

Abstract

Epidemiological studies suggest neurotoxicity in children after exposure to organophosphates pesticides (OP) at low levels but possible mechanism is still unclear. We aimed at investigating the effects of prenatal exposure to OPs on inhibitory control of 10 to 12 year-old-children assessed by a motor inhibition task during functional magnetic resonance imaging (fMRI). Ninety-five children from the PELAGIE cohort (Brittany-France, from 2002) underwent an fMRI examination during which inhibition was assessed by a Go/No-Go task. Task performance was assessed by average response latency, commission rate and composite performance score (PS). Whole brain activation was estimated by modeling the hemodynamic response related to inhibition demand and successful inhibition. OP exposure was assessed by measuring six dialkylphosphate (DAP) metabolites in the urine of women in early pregnancy (<19 WG). Concentrations were summed to obtain overall levels of diethylphosphate (DE), dimethylphosphate (DM) and total non-specific metabolites (DAP), standardized to homogenize sampling conditions and categorized into levels of exposure: low (reference), moderate or high. Regression models were adjusted for potential cofounders considered by restriction and statistical criteria. Moderate levels of DAP were associated with a decreased commission rate (β = -6.65%, p = 0.04), without increased PS. Increasing levels of DM and DE were associated with decreased brain activity in the left inferior and bilateral superior frontal regions during successful inhibition. We did not observe any differential activation related to inhibitory demands. These results suggest that OPs may be associated with altered pattern of brain activity in regions related to inhibition.

Introduction

Organophosphate pesticides (OPs) have been used worldwide since the 1970's for agricultural and pest control purposes due to their high acute neurotoxicity for insects and shorter environmental persistence than that of the organochlorine insecticides they substituted. Their half-life is limited from a few days to several months depending on the environmental conditions. However, they are frequently found, as parent compounds or degradation products, in both outdoor and indoor French environments (1, 2). A common exposure pathway of the general population is thought to be the ingestion of food or drinking water contaminated with organophosphate residues. A study of the typical French diet among pregnant women in 2011 estimated the daily intake of OP residues (including chlorpyrifos) to be sufficient to present a potential risk of neurochemical effects (3). Other sources of exposure including the use of household insecticides or living close to areas where OP insecticides are used for agriculture, are very likely in France (2). Since the 2000's, various mixtures of OPs or their metabolites have been frequently found in human biomonitoring studies, in particular in the urine or hair matrices of pregnant French women and children, suggesting widespread and repeated OP exposure (4–6).

The primary neurotoxic property of organophosphate compounds for insect control is the inhibition of acetylcholinesterase (AChE) activity in synaptic junctions, resulting in hyper-excitability of the nervous system. Similar effects have also been described in humans after accidental acute exposure. Other neurotoxic mechanisms of OPs have then been described at low doses including non-cholinergic effects, such as oxidative stress, neuro-inflammation, the alteration of axonal transport and mitochondria metabolism, the loss of neurons and glia, and persistent serotonergic effects (7–9).

Low-dose exposure is a matter of concern, especially during prenatal and childhood periods. Biomonitoring studies have reported organophosphate metabolites to be found in the placenta, cord blood and meconium matrices, suggesting the ability of OPs to cross the placental barrier (10, 11). Given the vulnerability of the developing blood-brain-barrier especially during fetal life (12–14), the epidemiological literature investigating potential adverse neurodevelopmental effects following prenatal exposure to OPs is extensive.

Although a variety of outcomes has been investigated, several studies have reported adverse effects on specific cognitive and behavioral functions that suggest possible alteration of executive functions. Most of published studies used neuropsychological tests or standardized questionnaires. We assume that the investigation of functional brain processes while children are engaged in inhibitory cognitive processes, suspected to be affected by OPs, has the potential to provide mechanistic insights into such processes. Task-related functional magnetic resonance imaging (fMRI) is one way to measure cerebral

activity during cognitive activity (15). A recently published pilot study used functional near-infrared spectroscopy (fNIRS) to investigate neural activity in Mexican-American adolescents from the CHAMACOS cohort (16). FNIRS is based on the same technique as fMRI, in which changes in neurovascular coupling during cerebral activity are detected. They reported associations between prenatal exposure to OP and altered activation pattern during tasks involving executive functions and language comprehension. There was a bilaterally decreased activity in the inferior frontal cortex during tasks of cognitive flexibility and working memory, which are two executive functions.

Among executive functions, inhibitory control is critical for learning (17) and difficulties in the ability to control and inhibit a prepotent response can lead to various developmental challenges, such as ADHD (18) and poor academic performance (19). Our aim was to assess the association between prenatal exposure to OP evaluated by maternal urinary biomarkers, and inhibitory control of 10- to 12-year-old children from a population-based mother-child cohort using fMRI during a motor inhibition task.

Materials and Methods

Study Population

The PELAGIE (*Perturbateurs endocriniens: Etude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance*) is a population-based mother-child cohort which included 3,421 women before 19 weeks of gestation from three districts of Brittany (France) between 2002 et 2006 (20). They were recruited by obstetricians, gynecologists, and ultra-sonographers at early visits for prenatal care. At inclusion, they completed a self-administered questionnaire about family, social, and demographic characteristics, diet, and lifestyle and sent a urine sample to the laboratory. Questionnaires given when their children were two and six years old allowed recording further information about the lifestyle, development and health of the child.

A sub-cohort of 251 children between 10 and 12 years of age was randomly selected for the present study to include approximatively 100 children. Children had to be born at term (delivery ≥ 35 weeks of amenorrhea (WA)) and present no major condition at birth (neonatal hospitalization, hypoglycemia, or five-minute Apgar score < 7), nor prenatal exposure to tobacco, alcohol, or medical treatment during childhood which could affect neurodevelopment (methylphenidate, psychotropic, or antiepileptic drugs, etc.) (5). Among them, 124 (49.4%) families refused to participate or were lost to follow-up, 26 (10.3%) were excluded due to technical (braces) or medical (meningitis, or head trauma requiring medical supervision) reasons. Thus, 101 (40.2%) children participated in the neuropsychological and fMRI examinations. Urine samples were not available for six women because they were used for other

urinary assays, resulting in 95 mother-child pairs for our study population (inclusion flowchart is shown in Figure I).

At the time of follow-up, parents completed a questionnaire about the environmental conditions and health of their child. All parent and child participants provided written informed consent and the appropriate ethics committees approved the study.

Organophosphate insecticide exposure assessment

At inclusion, women returned a urine sample (first morning void) in a 10−mL test tube (95 × 16−mm polypropylene, with wing plug). Six nonspecific dialkylphosphate (DAP), metabolites of numerous OPs were analyzed: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethyl-phosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). Analyses were performed in 2007-2008 (n = 54), 2013 (n = 22), and 2017 (n = 19) by liquid chromatography-electrospray ionization tandem mass spectrometry (LC/ MS-MS) after solid-phase extraction (SPE) at the LABOCEA (Laboratoire public Conseil, Expertise et Analyse en Bretagne). Details of the chemical analysis procedures have been previously described (5).

The limits of quantification (LOQ) for the chemical analyses performed in 2007-2008 (hereafter referred as series n°1) were 1.25, 1.7, 0.02, 0.2, 1, and 0.45 µg/L for DEP, DETP, DEDTP, DMP, DMTP, and DMDTP, respectively. Values between the limit of detection (LOD) and the LOQ were available for series n°2 and n°3 analyzed in 2013 and 2017, respectively. The LODs were 0.2, 0.1, 0.005, 0.06, 0.32, and 0.13 µg/L for DEP, DETP, DEDTP, DMP, DMTP, and DMDTP, respectively. The mass concentrations were converted to molar concentrations using the molecular masses (g/mol) from Toxnet database: DEP (CAS number 598-02-7) 154.101, DETP (CAS number 2465-65-8) 170.168, DEDTP (CAS number 52857-42-8) 186.235, DMP (CAS number 813-78-5) 126.0473, DMTP (CAS number 59401-04-6) 142.114, and DMDTP (CAS number 32534-66-0) 158.181. Concentrations were summed to obtain overall concentrations of diethylphosphate metabolites (DE: DEP, DETP, DEDTP), dimethylphosphate metabolites (DM: DMP, DMTP, DMDTP), and all nonspecific dialkylphosphate metabolites (DAP).

Motor inhibition function

The Go/No-Go paradigm

The motor inhibition function of the children was evaluated using a 10-minute visual Go/No-Go task completed in the MR scanner. First, a short practice run in front of a computer and then in a mock MR scanner was performed to provide the instructions and accustom the child to the scanner environment.

The event-related task, adapted from Mostofsky et al. 2003, minimizes cognitive demands other than motor execution and response inhibition and can be easily performed in children (22). Green and red smileys were rapidly presented on a screen, every 1.5 s. Children were asked to press a button as quickly as possible when seeing a green smiley but had to refrain otherwise. The ratio of No-go cues (red smileys) over Go cues (green smileys) was 1:4, to elicit a dominant motor response. The task was implemented using E-Prime v.2.0.8 Professional (Psychology Software Tools, Pittsburgh, PA, USA) and presented using the Nordic Neurolab Solution (Nordic Neurolab, Bergen, Norway).

The task was split into two runs of five minutes each, composed of 150 trials (123 Go and 27 No-Go cues) and four 10-second rest periods a run. A trial consisted of the consecutive presentation of a cue (duration of 200 ms) and a cross fixation point (duration of 1,300 ms). A response was allowed until the next trial began, giving a constant inter-stimulus interval of 1,500 ms. Smileys appeared in a pseudo-random order: cues at the beginning of runs or following resting periods had to be Go cues. Go cues had to occur at least three times in a row and No-Go cues had to occur at most two times in a row.

Performance indicators at the Go/No-Go task

Child performance was evaluated by response latency (average reaction time for the correct answering of Go cues), commission rate (incorrect answers for the No-Go cues), and a composite performance score. Responses occurring during cue presentation (before 200 ms) were considered anticipatory and were excluded from the indicator calculation (on average, less than 1% of all cues). The accuracy of motor inhibition was assessed by the sensitivity index, *d'*, which subtracts the standardized (z score) commission rate from the standardized hit rate (correct answers for Go cues). The response latencies and commission errors inversely correlated with each other (Spearman rho coefficient =-0.31, $p < 0.01$). Thus, we built a composite performance score by subtracting the response latencies from the accuracy of motor inhibition (*d'*), following standardization (23).

Thus, children with the high scores were considered to efficiently perform the task (fast with a high hit rate, with few commissions), whereas children with the lowest scores were considered to be slow and found it difficult to inhibit their response to No-Go cues. Performance indicators were missing for two children (resulting in n = 93 included children), due to technical issues with the recording.

Cerebral hemodynamic response related to the task

Scanning was performed on a 3 T MR Scanner (Magnetom Verio, VB17, Siemens, Erlangen, Germany) using a 32-channel receiver head coil.

High-resolution 3D anatomical images were obtained using T1-weighted MPRAGE at 1 mm^3 resolution for anatomical referencing (repetition/inversion/echo times: 1,900/900/2.26 ms, 9° flip angle).

Functional images were acquired using gradient echo-planar imaging (EPI) with repetition/echo times of 2,500/30 ms, a 90° flip angle, and 110 volumes per run. Each volume was composed of 34 axially oriented 4 mm-interleaved slices, covering the entire brain. Scans were based on a 110*110 acquisition matrix (220x220 mm^2 field of view), with a voxel size of 2*2*4 mm^3. Operators assessed image quality during the acquisition and two children who moved repeated the sequence. Learning effects are not expected for this task and we did not observe any improvement in performance during the two sessions.

Image pre-processing is described in Supplementary Material. Head movements were evaluated by six parameters calculated during the realignment step. Three children had head motion greater than 2 mm (in-plane size of an acquisition voxel) and they were excluded from further image analysis (resulting in n = 92 included children).

The blood oxygen level dependent (BOLD) response was estimated from trials (Go cue and No-Go cue) and motion regressors, with an implicit baseline corresponding to resting periods (enabling recovery of the hemodynamic response) and the time between trials, using a canonical hemodynamic response function (HRF) and its temporal derivative. This HRF is the subtraction of two gamma functions, the first modelling the peak of intensity, with a latency of 6 s, and the second one the undershoot during the recovery period, with a latency of 16 s. The use of a temporal derivative allows for variations in peak latency, while providing comprehensive models for the response. Scanner drift was modeled with a discrete cosine transform (DCT) set (128-second cut off) and temporal autocorrelation was accounted for using an autoregressive AR(1) model over the entire brain. First, we modeled Go cues and No-Go cues, independently of the subject's response. Secondly, we modeled successful No-Go, Go, and failed No-Go conditions. Maps were extracted for each condition and each subject by voxel-wise multiple linear regressions estimated by the restricted maximum likelihood (ReML) method and then contrasted. We built two types of individual contrast images, as we were investigating the ability to stop a planned response when it is no longer pertinent. First, we extracted “No-Go vs Go” activations, representing activation amplitudes that were higher for inhibition than motor tasks, when children perceived the inhibition demand. Secondly, we built contrast images for “Successful No-Go vs successful Go”, when children were able to inhibit and stop their answers, expected to reflect successful inhibition. There were no differences between the two runs for contrast estimates (at the uncorrected cluster level $p < 0.001$) or any performance indicators (at $p < 0.05$), allowing concatenation of the two runs.

215 Statistical analysis strategy

216 *Standardization of sampling conditions and imputation*

217 Sampling conditions of urinary samples of the present study are heterogeneous, in particular duration
218 of storage due to three different chronological series of analyses (2007-2008, 2013 and 2017).
219 Biomarker concentrations may vary according to sampling conditions. Thus, we aimed to standardize
220 the urinary concentrations of DAP under various sampling conditions to limit their possible influence
221 in the association study.

222 We adapted a standardization method based on regression residuals previously described by
223 Mortamais *et al.* (24). First, we estimated the influence of sampling conditions (sampling day, duration
224 of storage before and after freezing, and creatinine levels) using an adjusted Tobit regression model
225 to account for multiple censored data. We adjusted the Tobit models for the year and season of
226 sampling, as these parameters may be associated with the *true* level of exposure and reflect temporal
227 variation of OP uses and exposure. Then, left-censored data (values below the LOD) were imputed
228 from a log-normal distribution (25). Last, imputed concentrations were standardized using Tobit model
229 estimates as if urines had been sampled under the same conditions (i.e., week-day collection, freezing
230 before 24h after collection, duration of storage < 5 years and median creatinine concentration).

231 All OP metabolite concentrations used in further statistical analyses were imputed for values below
232 the LOD or LOQ and standardized for sampling conditions (sampling day, duration of storage and
233 creatinine levels) unless specified otherwise. DAP levels were categorized into three groups: for DAP
234 and DM in terciles, and for DE (with > 33% of values < LOD), values < LOD, values > LOD, and < median,
235 values > median. For clarity, we refer hereafter to low, moderate, or high levels of exposure.

236 *Statistical analyses*

237 Multivariable linear regression models were built on performance indicators (log-transformed-
238 response latency, commission rate, and performance score) to analyze the association with each
239 categorical level of OP metabolite. We used restricted cubic splines with log-transformed metabolite
240 concentrations to assess a possible dose-response relation. When checking for the assumption of
241 linearity, we plotted urinary metabolite levels as continuous.

242 The BOLD response during the motor inhibition task was modeled by mixed effect generalized linear
243 regression models to investigate the effect-modification of the prenatal exposure to OP by the motor
244 inhibition task on cerebral activity. We did not have any *a priori* hypotheses on brain regions that could
245 be differentially activated during the inhibition task across exposure levels and thus performed whole-
246 brain analyses. Certain regions are known to be part of the motor inhibition network (frontal and
247 anterior cingular cortices and supplementary motor area (SMA)) regions (21, 22). Thus, we focused on

these regions. Statistical significance was assessed for cluster-wise significance, defined by random field theory (FWER corrected p = 0.05) to account for 3D spatial autocorrelation, using an uncorrected one-sided cluster-defining threshold of p = 0.01 (26).

Then, we applied our linear regression models to all the children stratified by sex to investigate whether the association between OP metabolite levels and motor inhibition differ according to the sex of the child.

Potential cofounders

Potential adjustment variables were selected from lifestyle and socioeconomic variables collected at inclusion or during follow-up (i.e. maternal age, maternal educational level, pre-pregnancy BMI, pre-pregnancy tobacco consumption, fruit and vegetable consumption and fish consumption during pregnancy, child's sex, duration of breastfeeding, parity, child's educational level and child's lateralization at follow-up). They were included in all regression models if they were associated (at $p < 0.10$) with both OP metabolite levels and motor inhibition performance (see Supplementary Material, Table S.3); only breastfeeding (≤ vs > 3 months) met the criteria. Fruit and vegetable intake was also forced in all regression models (< *vs* ≥ 3 portions a day), as this has been described to be a possible source of DAP exposure (27).

Sensitivity analyses

Two sets of sensitivity analyses were performed. The first one excluded two children with extreme performance scores (statistical outliers outside averaged performance score ± 3* standard errors interval), and the second included OP metabolite levels imputed for values below the LOD but not standardized for sampling conditions.

R software v.3.5.1 (https://www.R-project.org/) and SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) were used for data analysis.

Results

Population characteristics

All pregnant women included in the study were of European origin (Table 1). At inclusion, they had a median age of 30.4 years, were highly educated (46.8% attended at least three years of post-secondary school) and declared to be employed (91.6%). Two thirds declared to be non smokers before inclusion. These women reported few medical problems during pregnancy (3.2% reported high blood pressure, no diabetes). They gave birth mainly to girls (56.8%), with a median birth weight of 3,430 g and 43.2% of the children were breastfed > three months. Their children at the follow-up time of the present study were mostly right-handed (87.4%), had a median age of 10.8 years, and most attended

elementary school (72.6%). We did not observe any differences between the study population and non-participants for these characteristics (see Supplementary material, Table S.1).

Urinary OP metabolite levels

OP metabolites were detected in the urine of 57% of the pregnant women for DE, 86% for DM and 95% for all DAP compounds with respective median values of 0.8, 31.8, and 44.4 µM (Table 1).

Increased creatinine concentrations were associated with increased levels of DE metabolites (1.10^{-4} µM for an increase of one µM of creatinine, $p = 0.02$). A moderate duration of storage before freezing (24-48 h) *versus* a short (< 24h) duration was associated with increased DM metabolite levels ($p < 0.01$) and a moderate duration of storage after freezing (5-10 years) *versus* a short (< 5 years) duration was associated with decreased DM metabolite concentrations ($p < 0.01$). We found no statistically significant association between sampling conditions and DAP metabolite levels nor difference with sampling day (weekday vs weekend) for any OP metabolite (see Supplementary Material, Table S.2). There was an association between analyses series and DAP metabolites ($\beta = -1.0$ for series 2 vs series 1 and $\beta = 0.3$ for series 3 vs series 1, global $p = 0.03$), for DM metabolites ($\beta = -2.6$ for series 2 vs series 1 and $\beta = 0.3$ for series 3 vs series 1, global $p < 0.001$) and not for DE metabolites ($\beta = 0.06$ for series 2 vs series 1 and $\beta = -0.34$ for series 3 vs series 1, global $p = 0.91$) (see Supplementary Material Figure S.I and Table S.2).

As expected, differences did not remain statistically significant after standardization of sampling conditions (for DAP, $p = 0.82$; for DM, $p = 0.23$) (see Supplementary Material Figure S.I). The median standardized OP metabolite levels presented were 0.8 µM, 17µM, and 25 µM for DE, DM, and DAP, respectively (see Supplementary Material Table S.3).

Performance indicators and brain activation during the task

Children performed the Go/No-Go task with a median commission rate of 25.9%, and reaction time of 399.6 ms. The performance score ranged from -9.8 to 2.1, with a median of 0.3 (see Supplementary Material Table S.3).

For “No-go vs Go” and “Successful No-Go vs successful Go” contrasts in the brain, children showed activation in regions known to be involved in the motor inhibition network: the anterior cingulate/SMA and inferior and middle frontal regions of both hemispheres. These contrasts were also associated with activated clusters in the right middle temporal region and brain structures of both hemispheres (occipital and parietal region, posterior lobe of the cerebellum, caudate nucleus, and putamen) (see Supplementary Material, Table S.4 and Table S.5).

Prenatal urinary organophosphate metabolite levels and motor inhibition

There was an association between moderate levels of DAP and decreased commission rate (β = -6.65%, 95% confidence interval (CI): -12.83; -0.48), without a statistically significantly higher performance score (β = 0.33, CI: -0.49; 1.15). There was a trend towards a lower performance score (β = -0.76, CI: -1.56; 0.03) and longer response latency (β = ($e^{0.08}$-1)*100 = 8%, CI: 0; 16) associated with moderate DE levels. Children whose mothers had moderate urinary levels of DM metabolites had a lower commission rate (β = -5.59), but the association was not statistically significant (CI: -11.71; 0.53) (Figure II). Our results did not show a linear dose-response relation between any child's inhibition performance indicators and maternal urinary OP metabolite levels when OP metabolite concentrations were used as continuous variables (Figure II and Supplementary material Figure S.II). The associations were similar for children of either sex, with no difference in the estimates, except for commission rate and moderate DE levels (β = 8.23, CI: -0.17; 16.63 for girls (n = 53) and β = -7.33, CI: -15.98; 1.33 for boys (n = 40)).

The first set of sensitivity analyses, which excluded statistical outliers, confirmed the previous statistically significant associations observed between commission rate and moderate DAP levels. Most of the associations were similar in the second set of sensitivity analyses when the OP metabolite levels were not standardized for sampling condition, except that response latency was significantly higher for children whose mothers showed high DE urinary levels (β = 8%, CI: 0 ; 16) (see Supplementary material Tables S.4 and S.5).

We did not observe any differential activation related to inhibition demand perception. We did not observe any difference in the inhibition network, stratified by sex, except for girls, with decreased brain activity in the medial superior frontal region in association with moderate levels of DM metabolites see Supplementary Material, Table S.6).

When children succeeded in inhibiting their response, there was decreased activation in the left inferior frontal regions associated with high urinary levels of DM metabolites. Children whose mothers had moderate or high levels of DE metabolites exhibited a lower BOLD response in the left and right frontal regions (Figure III). We did not observe any differential brain activation in girls when stratifying the analyses by sex. However, we observed decreased brain activity in the frontal regions of boys whose mothers had high levels of DAP or DM metabolites or moderate urinary levels of DE metabolites.

Discussion

The present study investigated the potential neurodevelopmental toxicity of prenatal exposure to OPs on motor inhibition function in a Brittany population-based cohort of children. We did not observe any

major alterations of the children's performance on the Go/No-Go task. A decreased commission rate associated with moderate levels of DAP suggests better performance. There was no differential brain activation related to inhibitory demands, except for decreased cerebral activity related to successful inhibition in regions involved in the motor inhibition network.

Previous epidemiological studies investigating the neurodevelopmental effects of prenatal exposure to OP have suggested possible alterations in children's inhibitory control. In a recent pilot study, Sagiv *et al.* reported an association between prenatal exposure to OPs and altered patterns of brain activity during tasks of executive function and language comprehension using the fNIRS technique on 95 adolescents from a population of agricultural communities of the Salinas Valley in California (CHAMACOS study). They found null associations during the Go/No-Go task but reported decreased bilateral brain activation in the prefrontal cortex during a cognitive flexibility task (16). In the CHAMACOS study, prenatal exposure to OP was also assessed by two maternal urinary samples collected during the first and second halves of pregnancy. The mothers had higher levels of DAP (109 nM DAP, 17.7 nM DE and 76.8 nM DM) than the present study. The authors reported statistically greater attention problems, ADHD, and K-CPT ADHD Confidence Index scores for five-year-old children (n = 323) and a similar but non-significant association at 3.5 years of age (28). Furlong *et al.* investigated the association between prenatal exposure to OP and neurodevelopmental phenotypes in six- to nine-year-old children (n = 141) at the Mount Sinai Children's Environmental Health Center. Exposure was assessed by analyzing maternal urine collected between 25 and 40 weeks of gestation. Urinary levels were higher than in the PELAGIE cohort (median of 37.1 nM DM and 16.6 nM DE). They reported positive associations between DM metabolite levels and executive functioning factor scores. The impulsivity and externalizing factor score was not associated with maternal urinary levels of DE or DM (29). A cross-sectional study investigated the effect of prenatal and postnatal exposure to OP insecticides on the neurodevelopment of six- to eight-year-old Ecuadorian primary school children (n = 84) and reported a borderline significant association between reaction time and attention test scores and the children's current exposure (30). A cross-sectional study evaluated the effects of methyl-parathion (MP) exposure during childhood on six-year-old-children living in Mississippi and Ohio. Exposed children (n=132) were more prone to attention, and behavioral skill problems than controls (n = 147). However, the results were not consistent between the two sites of the study (31). Although they were not specifically investigating inhibition function, the results of Rauh *et al.* showing that seven-year-old children with high prenatal exposure to chlorpyrifos had frontal cortical thinning, also suggest brain alterations in the frontal cortex, involved in inhibition (32). Findings of brain alterations after prenatal exposure to OPs are supported by one animal study that exposed gestational dams to 5mg/kg/day chlorpyrifos during gestational days 13 to 17. At postnatal day 60, the authors reported

reduced neuron and glia count in anterior cingulate, prelimbic, and infralimbic areas of medial prefrontal cortex (mPFC), a region of the inhibition network, at levels showing no effect on body weight, organ coefficient, or pathological morphological changes (such as cytoplasm swelling, enlarged intercellular spaces, or cell apoptosis) in the mPFC (7).

Two major types of inhibitory control can be distinguished, cognitive and behavioral inhibition (17). The task we used involves behavioral response inhibition with a motor component. Response inhibition appears in early childhood and matures until adolescence (33). Performance indicators in our population of 10- to 12-year-old children were similar to those reported during a similar Go/No-Go task with 8- to 13-year-old children (median response latency of 400 ms *vs* 407 ms and 25.9% *vs* 22% for commission errors) (22). We did not observe any differential performance score for the Go/No-Go task but observed decreased brain activity in frontal regions, known to be involved in inhibitory control. Children who are the most highly exposed to OP during pregnancy may present compensatory brain functioning to prevent problems of inhibition. However, we cannot eliminate the possibility that our neuropsychological indicators did not have sufficient sensitivity to discriminate the inhibitory capacity between the children.

The motor inhibition network concerns three main regions: the anterior cingulate, SMA, and inferior frontal gyri of both hemispheres (21, 34). As expected, we observed cerebral activation in these regions in the contrasts that we explored. We also observed additional activation in other brain structures of both hemispheres (occipital and parietal region, posterior lobe of the cerebellum, caudate nucleus and putamen). These regions are not part of the motor inhibition network but have been described to be involved in certain motor inhibition tasks, in particular in 8- to 13-year-old children (22). Activation observed in these brain areas may reflect ancillary processes necessary for the Go/No-Go task, such as vision processing, attention and decision making (35–38). Nevertheless, BOLD imaging measures the ratio of deoxyhemoglobin to oxyhemoglobin to indirectly evaluate neural activity. This indicator is commonly used, but may vary depending on individual characteristics (blood flow, iron deficiency, etc.) and the performance of inhibition is not determined solely by the intensity of the hemodynamic response but could also be explained by brain structure, connectivity, etc. (39). Reduced activation may indicate that exposure altered the recruitment of neural resources, such as a lack of neural fibers and/or reduced connectivity of the inhibitory network. The associations observed between prenatal levels of DM and DE metabolites and decreased activity from different regions of the brain may suggest an alteration in the pattern of brain activity.

We observed no sex-specific effects on performance indicators but observed decreased brain activity related to successful inhibition only in boys. This finding is consistent with the literature, which

411 suggests that boys may be more sensitive to OP exposure (28, 40). In their review, Comfort and Re
412 reported that sex-specific effects of OP exposure have been commonly found in rodent studies.
413 Whereas, the exact mechanisms of sex-dependent OP effects are not fully established, authors
414 assumed it could be the combination of altered morphology, neuropeptide and neurotransmitter
415 signaling, and neuroinflammation. They highlighted the necessity to investigate this effect in
416 epidemiological studies. Indeed, Marks *et al.* suggested an increased risk of ADHD at five years of age
417 in the CHAMACOS cohort in association with prenatal exposure to OP in boys only.

418 Our study was based on a mother-child cohort (PELAGIE study) in which the longitudinal design
419 provides the opportunity to measure OP insecticide exposure during early pregnancy. We were also
420 able to consider lifestyle and socioeconomic factors by restriction or adjustment. In particular, children
421 whose mothers reported alcohol or tobacco consumption during pregnancy were not included in the
422 study to eliminate two established risk factors of poorer neurodevelopment or behavioral troubles in
423 children (41). We assessed prenatal exposure to OPs by maternal urinary biomarkers, which gives us
424 the ability to capture all sources of exposure. We used fMRI to investigate brain functioning during an
425 inhibition task in association with prenatal exposure to OP in the general population. This technique
426 provides excellent spatial resolution, making it possible to investigate the potential effects of exposure
427 in brain regions of only a few cubic millimeters.

428 Although we did not find any differences in the characteristics of our study population and non-
429 participants, limiting the risk that a confounder may predict participation, the design of the PELAGIE
430 cohort does not prevent a possible selection bias. Unlike the previously described epidemiological
431 studies, the PELAGIE population does not include an ethnic minority and consists of participants with
432 a high socioeconomic status. We cannot rule out the possibility that the stimulating environments and
433 possible healthier lifestyles in our study population attenuated the potential neurotoxicity of OP
434 exposure. The main limitation of our study was the exposure assessment, based on only one (first)
435 morning void urinary sample collected during the first trimester of pregnancy, whereas OP insecticides
436 have a short half-life, from several hours to several days (42). It is likely that our measurement did not
437 capture intra-individual variability, over time, due to the rapid metabolism and excretion of OP
438 insecticides. This produces non-differential measurement errors in the exposure estimation. We could
439 not explore the effects of OP exposure during late pregnancy or childhood, although human
440 neurodevelopment is known to continue during childhood, until adolescence for maturation of the
441 prefrontal cortex (14). A recent study in the Netherlands, showing few effects of prenatal exposure to
442 OP on nonverbal IQ in six-year-old children, suggests that late pregnancy may be a susceptible window
443 for adverse effects on cognition (43). DAP metabolites are found in food and dust. Thus, measurements

444 of urinary levels of DAP may reflect intake of both the toxic parent OP compound and the metabolite
445 itself, without possible distinction and biased exposure classification (27).

446 Conclusion

447 We found no major alteration of neuropsychological scores in a French population-based cohort of 10
448 to 12 year-old-children with a high socioeconomic status, but found decreased brain activity in areas
449 related to inhibition in association with prenatal exposure to OPs. These findings need to be confirmed
450 by other studies and more deeply investigated by further neuroimaging techniques of morphometry,
451 and functional or structural connectivity.

References

1. C. Coscollà, *et al.*, Human exposure and risk assessment to airborne pesticides in a rural French community. *Science of The Total Environment* **584–585**, 856–868 (2017).

2. G. Raffy, *et al.*, Semi-volatile organic compounds in the air and dust of 30 French schools: a pilot study. *Indoor Air* **27**, 114–127 (2017).

3. E. de Gavelle, *et al.*, Chronic dietary exposure to pesticide residues and associated risk in the French ELFE cohort of pregnant women. *Environment International* **92–93**, 533–542 (2016).

4. R. Béranger, *et al.*, Multiple pesticide analysis in hair samples of pregnant French women: Results from the ELFE national birth cohort. *Environment International* **120**, 43–53 (2018).

5. C. Cartier, *et al.*, Organophosphate Insecticide Metabolites in Prenatal and Childhood Urine Samples and Intelligence Scores at 6 Years of Age: Results from the Mother–Child PELAGIE Cohort (France). *Environ Health Perspect* **124**, 674–680 (2016).

6. C. Dereumeaux, *et al.*, Biomarkers of exposure to environmental contaminants in French pregnant women from the Elfe cohort in 2011. *Environment International* **97**, 56–67 (2016).

7. X. P. Chen, Y. S. Chao, W. Z. Chen, J. Y. Dong, Mother gestational exposure to organophosphorus pesticide induces neuron and glia loss in daughter adult brain. *Journal of Environmental Science and Health, Part B* **52**, 77–83 (2017).

8. T. A. Slotkin, F. J. Seidler, The alterations in CNS serotonergic mechanisms caused by neonatal chlorpyrifos exposure are permanent. *Developmental Brain Research* **158**, 115–119 (2005).

9. A. V. Terry, Functional consequences of repeated organophosphate exposure: Potential non-cholinergic mechanisms. *Pharmacology & Therapeutics* **134**, 355–365 (2012).

10. M. K. Silver, *et al.*, Prenatal naled and chlorpyrifos exposure is associated with deficits in infant motor function in a cohort of Chinese infants. *Environment International* **106**, 248–256 (2017).

11. Whyatt R M, Barr D B, Measurement of organophosphate metabolites in postpartum meconium as a potential biomarker of prenatal exposure: a validation study. *Environmental Health Perspectives* **109**, 417–420 (2001).

12. B. Eskenazi, A. Bradman, R. Castorina, Exposures of children to organophosphate pesticides and their potential adverse health effects. *Environmental Health Perspectives* **107**, 409–419 (1999).

13. A. Gupta, R. Agarwal, G. S. Shukla, Functional impairment of blood-brain barrier following pesticide exposure during early development in rats. *Hum Exp Toxicol* **18**, 174–179 (1999).

14. D. Rice, S. Barone, Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect* **108**, 511–533 (2000).

15. M. K. Horton, A. E. Margolis, C. Tang, R. Wright, Neuroimaging is a novel tool to understand the impact of environmental chemicals on neurodevelopment. *Curr Opin Pediatr* **26**, 230–236 (2014).

16. S. K. Sagiv, *et al.*, Prenatal exposure to organophosphate pesticides and functional neuroimaging in adolescents living in proximity to pesticide application. *PNAS*, 201903940 (2019).

17. A. Bari, T. W. Robbins, Inhibition and impulsivity: behavioral and neural basis of response control. *Progress in Neurobiology* **108**, 44–79 (2013).

18. E. G. Willcutt, A. E. Doyle, J. T. Nigg, S. V. Faraone, B. F. Pennington, Validity of the Executive Function Theory of Attention-Deficit/Hyperactivity Disorder: A Meta-Analytic Review. *Biological Psychiatry* **57**, 1336–1346 (2005).

19. A. Diamond, W. S. Barnett, J. Thomas, S. Munro, Preschool Program Improves Cognitive Control. *Science* **318**, 1387–1388 (2007).

20. R. Garlantézec, C. Monfort, F. Rouget, S. Cordier, Maternal occupational exposure to solvents and congenital malformations: a prospective study in the general population. *Occupational and Environmental Medicine* **66**, 456–463 (2009).

21. S. H. Mostofsky, *et al.*, fMRI evidence that the neural basis of response inhibition is task-dependent. *Cognitive brain research* **17**, 419–430 (2003).

22. S. J. Suskauer, *et al.*, Functional magnetic resonance imaging evidence for abnormalities in response selection in attention deficit hyperactivity disorder: differences in activation associated with response inhibition but not habitual motor response. *J Cogn Neurosci* **20**, 478–493 (2008).

23. O. Collignon, *et al.*, Women process multisensory emotion expressions more efficiently than men. *Neuropsychologia* **48**, 220–225 (2010).

24. M. Mortamais, *et al.*, Correcting for the influence of sampling conditions on biomarkers of exposure to phenols and phthalates: a 2-step standardization method based on regression residuals. *Environ Health* **11**, 29 (2012).

25. Y. Jin, M. J. Hein, J. A. Deddens, C. J. Hines, Analysis of Lognormally Distributed Exposure Data with Repeated Measures and Values below the Limit of Detection Using SAS. *Ann Occup Hyg* **55**, 97–112 (2011).

26. T. Nichols, S. Hayasaka, Controlling the familywise error rate in functional neuroimaging: a comparative review. *Statistical Methods in Medical Research* **12**, 419–446 (2003).

27. D. L. Sudakin, D. L. Stone, Dialkyl phosphates as biomarkers of organophosphates: The current divide between epidemiology and clinical toxicology. *Clinical Toxicology* **49**, 771–781 (2011).

28. A. R. Marks, *et al.*, Organophosphate Pesticide Exposure and Attention in Young Mexican-American Children: The CHAMACOS Study. *Environmental Health Perspectives* **118**, 1768–1774 (2010).

29. M. A. Furlong, *et al.*, Prenatal exposure to organophosphorus pesticides and childhood neurodevelopmental phenotypes. *Environmental Research* **158**, 737–747 (2017).

30. Harari Raul, *et al.*, Neurobehavioral Deficits and Increased Blood Pressure in School-Age Children Prenatally Exposed to Pesticides. *Environmental Health Perspectives* **118**, 890–896 (2010).

31. P. Ruckart, Kakolewski Kirsten, Bove Frank J, Kaye Wendy E, Long-term neurobehavioral health effects of methyl parathion exposure in children in Mississippi and Ohio. *Environmental Health Perspectives* **112**, 46–51 (2004).

526 32. V. A. Rauh, *et al.*, Brain anomalies in children exposed prenatally to a common organophosphate
527 pesticide. *Proceedings of the National Academy of Sciences* **109**, 7871–7876 (2012).

528 33. B. Luna, J. A. Sweeney, The Emergence of Collaborative Brain Function: fMRI Studies of the
529 Development of Response Inhibition. *Annals of the New York Academy of Sciences* **1021**, 296–
530 309 (2006).

531 34. A. R. Aron, The Neural Basis of Inhibition in Cognitive Control. *The Neuroscientist* **13**, 214–228
532 (2007).

533 35. M. Behrmann, J. J. Geng, S. Shomstein, Parietal cortex and attention. *Current Opinion in*
534 *Neurobiology* **14**, 212–217 (2004).

535 36. J. A. Grahn, J. A. Parkinson, A. M. Owen, The cognitive functions of the caudate nucleus. *Progress*
536 *in Neurobiology* **86**, 141–155 (2008).

537 37. M. L. Rosen, *et al.*, The Role of Visual Association Cortex in Associative Memory Formation across
538 Development. *J Cogn Neurosci* **30**, 365–380 (2018).

539 38. C. J. Stoodley, J. D. Schmahmann, Functional topography in the human cerebellum: A meta-
540 analysis of neuroimaging studies. *NeuroImage* **44**, 489–501 (2009).

541 39. S. Ogawa, T. M. Lee, A. R. Kay, D. W. Tank, Brain magnetic resonance imaging with contrast
542 dependent on blood oxygenation. *Proc Natl Acad Sci U S A* **87**, 9868–9872 (1990).

543 40. N. Comfort, D. B. Re, Sex-specific Neurotoxic Effects of Organophosphate Pesticides Across the
544 Life Course. *Curr Environ Health Rep* **4**, 392–404 (2017).

545 41. K. Yolton, *et al.*, Exposure to neurotoxicants and the development of attention deficit
546 hyperactivity disorder and its related behaviors in childhood. *Neurotoxicology and Teratology* **44**,
547 30–45 (2014).

548 42. L. G. Costa, Organophosphorus Compounds at 80: Some Old and New Issues. *Toxicol Sci* **162**, 24–
549 35 (2018).

550 43. T. A. Jusko, *et al.*, Organophosphate Pesticide Metabolite Concentrations in Urine during
551 Pregnancy and Offspring Nonverbal IQ at Age 6 Years. *Environmental Health Perspectives* **127**,
552 017007 (2019).

553

Table

Table 1: Characteristics of participants included in the study.

Characteristics	*n=95*	*Study population*
	N	%
Family characteristics		
European origin	95	100 %
Maternal characteristics		
Age		30.4 (28.1;33.5) [a]
<30 years old	41	43.2 %
30-35 years old	40	42.1 %
>35 years old	14	14.7 %
Body Mass Index		
<18.5 kg/m²	10	10.5 %
18.5-25 kg/m²	69	72.6 %
≥25 kg/m²	16	16.8 %
Post-secondary school		
2 years or less	50	53.2 %
≥ 3 years	44	46.8 %
Missing value	1	
Occupation at inclusion		
no	8	8.4 %
yes	87	91.6 %
Smoker before inclusion		
no	62	65.3 %
yes	33	34.7 %
High blood pressure		
no	92	96.8 %
yes	3	3.2 %
Diabetes		
no	94	100 %
Missing value	1	
Fruit and vegetable consumption		
<3 portions/day	78	82.1 %
≥3 portions/day	17	17.9 %
Fish consumption		
< twice a month	62	65.3 %
≥ twice a month	33	34.7 %
Newborn characteristics		
Sex		
Boys	41	43.2 %
Girls	54	56.8 %
Gestational age		
≤39 weeks of amenorrhea	44	46.3 %
>39 weeks of amenorrhea	51	53.7 %
Birth weight		3430 (3165;3800 [a]
<3000 g	12	12.6 %
≥ 3000 g	83	87.4 %
Breastfeeding		2 (0;5.7)
≤ 3 months	54	56.8 %
> 3 months	41	43.2 %
Child characteristics (at follow-up)		
Parity		
single child	16	16.8 %
1 sibling	43	45.3 %
≥ 2 siblings	36	37.9 %
Age		10.8 (10.6;11.1) [a]
10 years old	66	69.5 %

11 years old	29	30.5 %
Educational level		
elementary school	69	72.6 %
junior high school	26	27.4 %
Lateralization		
right-hander	83	87.4 %
left-hander	12	12.6 %

Note: DAP: Dialkylphosphate, DE: diethylphosphate, DM: dimethylphosphate

[a]: median (Q1; Q3)

Table 2: Associations between dialkylphosphate levels in maternal urines during pregnancy and activation intensity related to successful inhibition during the Go/No-Go task of their 10-12 years old children (PELAGIE cohort. 2002-2017) (FWER correction at p<0.05).

Metabolite levels	N	*Right frontal region*		*Left frontal region*		*Anterior cingular region*		*Other brain regions*	
		β (95% CI)	ext.	β (95% CI)	ext.	β (95% CI)	ext.	β (95% CI)	ext.
DAP									
Low levels	31	Ref.		Ref.		Ref.		Ref.	
Moderate levels	28								
High levels	31								
DM									
Low levels	38	Ref.		Ref.		Ref.		Ref.	
Moderate levels	25								
High levels	27			-1.2 (-1.6 ; -0.8)	465				
DE									
Low levels	32	Ref.		Ref.		Ref.		Ref.	
Moderate levels	29	-1.8 (-2.5 ; -1.1)	1072	-1.7 (-2.4 ; -1.0)	951				
High levels	29	-1.6 (-2.0 ; -1.2)	390						

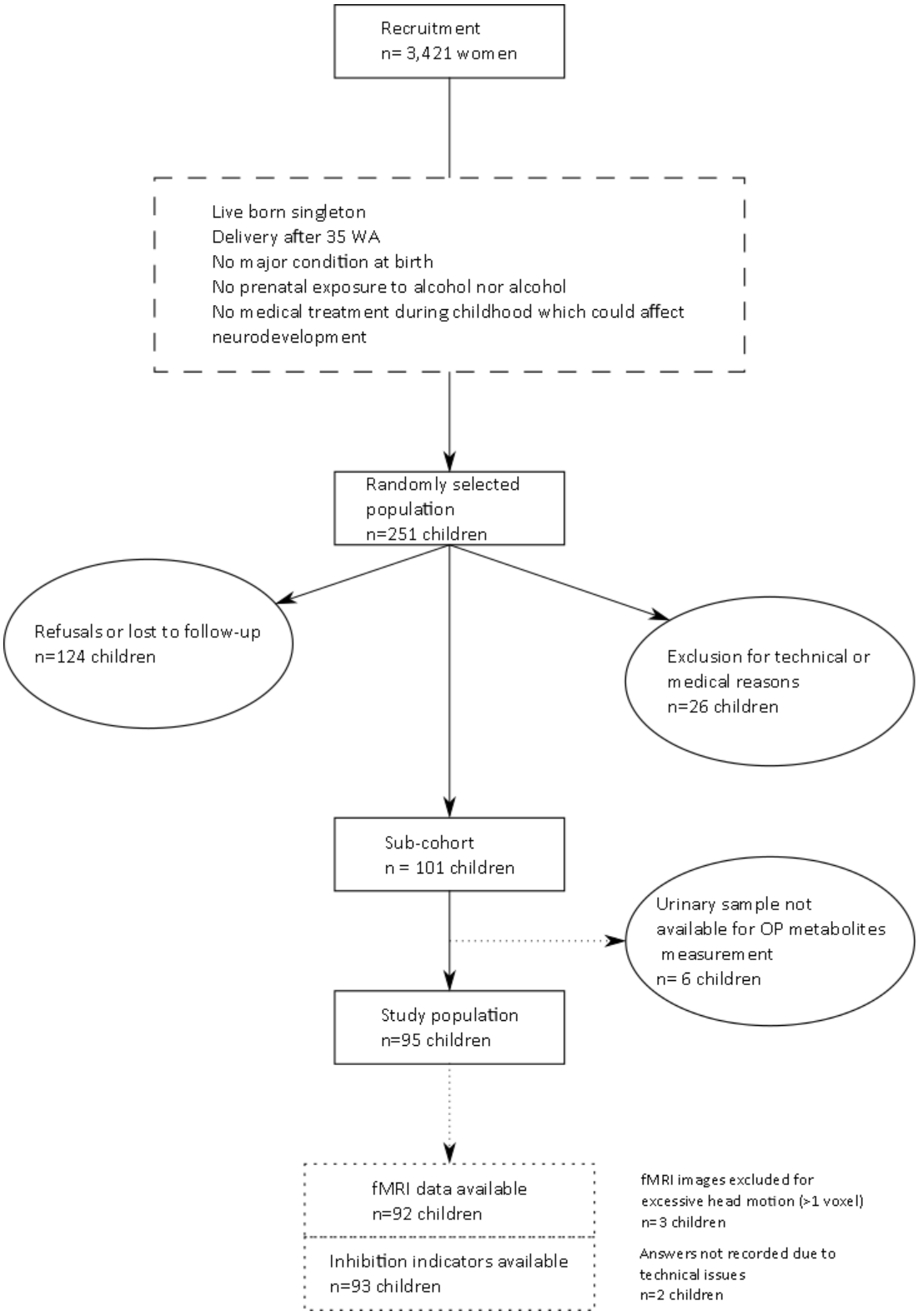
Note: DAP: Dialkylphosphate, DE: diethylphosphate, DM: dimethylphosphate

β (95% IC): estimated coefficient [95% confidence interval]. Empty cell: no statistically significant difference reported in this region.

All estimates are from mixed-effect generalized linear regression models adjusted for maternal fruit/vegetable consumption (< 3 portions a day vs ≥ 3 portions a day) and the duration of breastfeeding (< 3 months vs > 3 months).

Figure legends

Figure I: Flowchart of inclusions from the PELAGIE cohort (n=3,421) to the present study (n=95).Figure I. Flowchart of inclusions from the PELAGIE cohort (n=3,421) to the present study (n=95).



Note: WA: weeks of amenorrhea

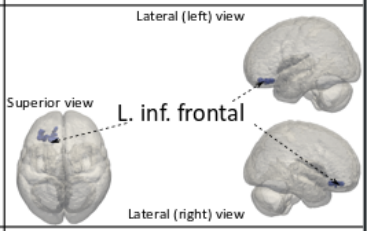
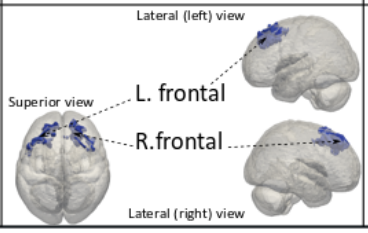
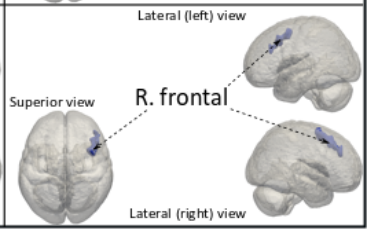
Figure II: Associations between OP metabolites levels in maternal urine during pregnancy and performance indicators for the Go/No-Go task of their 10- to 12-year-old children (PELAGIE cohort, 2002-2017) (n =92).

Note: DAP: dialkylphosphate, DE: diethylphosphate, DM: dimethylphosphate

All estimates are from linear regression models adjusted for maternal fruit/vegetable consumption (< 3 portions a day vs ≥ 3 portions a day) and the duration of breastfeeding (< 3 months vs > 3 months).

*$p < 0.05$, †: $0.05 \leq p < 0.10$.

Figure III: Summary of associations between urinary OP metabolites levels during pregnancy and brain activation during successful inhibition (n = 90)*.

	Low level	Moderate level (vs low)	High level (vs low)
DAP	Reference	*No statistically significant difference*	*No statistically significant difference*
DM	Reference	*No statistically significant difference*	
DE	Reference		

Note: DAP: dialkylphosphate, DE: diethylphosphate, DM: dimethylphosphate, inf.: inferior, L: left, R: right

Adjusted for maternal fruit/vegetable consumption (< 3 portions a day vs ≥ 3 portions a day) and the duration of breastfeeding (< 3 months vs > 3 months).

*FWER corrected-p ≤ 0.05 with a one-sided uncorrected-p ≤ 0.01 threshold.

Supplementary Material

Image pre-processing

Anatomical images were segmented into cerebrospinal fluid, grey and white matters, bone, soft tissues and air by using tissue probability maps. Head motion artifacts on anatomical images and segmented tissues were checked visually for each subject.

Functional images were first corrected for differences in acquisition time between slices and realigned with a six-parameter (rigid body) approach. A mean volume was created and coregistered to the subject high-resolution anatomical image.

A common group template was created using DARTEL from individual anatomical images and spatially normalized to the Montreal Neurological Institute (MNI) - labeled space. Functional images were normalized to the created DARTEL template and smoothed using a Gaussian kernel of 8 mm^3.

Image preprocessing was performed using Matlab v.9.2 (Mathworks. Natick. MA. USA) and SPM12 (http://www.fil.ion.ucl.ac.uk /spm/). DARTEL template was built using SPM8.

Table S. 1: Characteristics of study population (n=95) versus non-participants (n=156).

	Study population (N=95)		*Non-included population (N=156)*		
Caracteristics	N	%	N	%	pvalue
Family characteristics					
European origin	95	100 %	154	98.7 %	0.52
Other	0	0%	2	1.3 %	
Maternal characteristics					
Age					
<30 years old	41	43.2 %	75	48.1 %	0.7
30-35 years old	40	42.1 %	59	37.8 %	
>35 years old	14	14.7 %	22	14.1 %	
Body Mass Index					
<18.5 kg/m²	10	10.5 %	13	8.3 %	0.51
18.5-25 kg/m²	69	72.6 %	112	71.8 %	
≥25 kg/m²	16	16.8 %	31	19.9 %	
Post-secondary school					
2 years or less	50	53.2 %	93	59.6 %	0.33
≥ 3 years	44	46.8 %	63	40.4 %	
Missing value	1		0		
Occupation at inclusion					
no	8	8.4 %	14	9 %	0.87
yes	87	91.6 %	142	91 %	
Smoker before inclusion					
no	62	65.3 %	107	68.6 %	0.89
yes	33	34.7 %	49	31.4 %	
High blood pressure					
no	92	96.8 %	147	94.8 %	0.53
yes	3	3.2 %	8	5.2 %	
Missing value	0		1		
Diabetes					
no	94	100 %	149	96.8 %	0.08
yes	0	0%	5	3.2 %	
Missing value	1		2		
Fruit and legume consumption					
<3 parts/day	78	82.1 %	116	74.4 %	0.29
≥3 parts/day	17	17.9 %	40	25.6 %	
Fish consumption					
< twice a month	62	65.3 %	121	77.6 %	0.08

≥ twice a month	33	34.7 %	35	22.4 %	
Newborn characteristics					
Sex					
boys	41	43.2 %	70	44.9 %	0.97
girls	54	56.8 %	86	55.1 %	
Gestational age					
≤39 weeks of amenorrhea	44	46.3 %	57	36.5 %	0.2
>39 weeks of amenorrhea	51	53.7 %	99	63.5 %	
Birth weight					
<3000 g	12	12.6 %	25	16 %	0.61
≥ 3000 g	83	87.4 %	131	84 %	
Breastfeeding					
≤ 3 months	54	56.8 %	102	65.8 %	0.23
> 3 months	41	43.2 %	53	34.2 %	
Missing value	0		1		

Table S. 2 : Average OP metabolites levels among sampling conditions.

Characteristics	N	DAP[a] (median(Q1;Q3))	DM[a] (median(Q1;Q3))	DE[a] (median(Q1;Q3))
N (%)>LOD		90 (95 %)	82 (86 %)	54 (57%)
Series				
n°1 (in 2007-2008)	54	44.4 (17.3;74.2)	31.8 (12.7;64.6)	0.8 (<LOD;16.3)
n°2 (in 2013)	22	11.9 (2.1;119)	2.1 (<LOD;4)	0.7 (<LOD;60)
n°3 (in 2017)	19	49.1 (28.7;132.3)	49.1 (24.3;125.3)	2.5 (<LOD;10)
p value[b]		0.03	<0.01	0.60
Sampling day				
week day	67	36 (12;76.3)	20.7 (5.2;63.2)	0.5 (<LOD;15.7)
week-end	28	64 (26.6;101.4)	29.2 (3.2;67.2)	4.5 (<LOD;25.4)
p value[b]		0.19	0.97	0.16
Duration before freezing				
<24h	38	32.6 (10.7;72.7)	10.6 (2.4;44.8)	0.6 (<LOD;20.2)
24-48h	25	63.4 (27.1;117.2)	57.4 (22;81.9)	8.3 (0.4;21.9)
>48h	32	35.7 (11.2;74.9)	20.1 (2.4;64)	0 (<LOD;13.8)
p value[b]		0.20	<0.01	0.23
Duration after freezing				
<5 years	50	39.3 (16.4;67.9)	27 (12.7;63.5)	0.4 (<LOD;13.9)
5-10 years	26	18.4 (2.4;113.5)	2.4 (0.2;6.9)	5.6 (<LOD;60)
>10 years	19	49.1 (28.7;132.3)	49.1 (24.3;125.3)	2.5 (<LOD;10)
p value[b]		0.14	<0.01	0.43

Year of inclusion				
in 2003	58	20.1 (11.4;69.6)	9.6 (1.8;38.2)	0.8 (<LOD;17.9)
in 2004	24	43.9 (17.4;112.6)	29.2 (8.2;66.6)	2 (<LOD;19.4)
in 2005	13	58.3 (6.2;75.7)	23.8 (2.1;58.3)	1.4 (<LOD;14)
p value[b]		0.43	0.05	0.97
Season of inclusion				
in spring	25	44.1 (12.6;116.5)	36 (7;66)	0.4 (<LOD;16.4)
in summer	28	27.2 (9;80.6)	22.4 (2.2;67.7)	0.4 (<LOD;10.9)
in autumn	20	53.8 (19.4;164.8)	12.2 (2.5;46.7)	13.2 (<LOD;50.2)
in winter	22	39.3 (14.8;67.7)	29.9 (12.6;55.4)	0.2 (<LOD;15.5)
p value[b]		0.40	0.38	0.07

Note: DAP: Dialkylphosphate, DE: diethylphosphate, DM: dimethylphosphate, LOD: limit of detection

[a]: crude and non-imputed OP metabolites levels

[b]: chi² test from Tobit regression models for effect between sampling characteristics and OP metabolites levels

Table S. 3: Average OP metabolites levels and inhibition indicators by characteristics.

Characteristics	*n=95*	*OP metabolites levels[a] (median(Q1;Q3))*			*Performance indicators (median(Q1;Q3))*		
	N	DAP (in nM)	DM (in nM)	DE (in nM)	Score	Response latency (in ms)	Commission rate (in %)
Family characteristics							
European origin	95	25 (10.6;64.5)	17 (6.8;36)	0.8 (0.1;10.3)	0.3 (-0.4;0.8)	399.6 (367.6;425.5)	25.9 (16.7;35.2)
Maternal characteristics							
Age							
<30 years old	41	21.9 (10.3;49.4)	19.5 (9.9;33.8)	0.1 (0;5.2)	0.2 (-0.6;0.8)	403.8 (357.6;436.4)	25.9 (15.7;35.2)
30-35 years old	40	30.3 (13.5;97.8)	15.5 (6.2;47.7)	3.8 (0.1;14)	0.2 (-0.2;0.7)	408 (378.2;423.1)	22.2 (16.2;35.2)
>35 years old	14	15 (4;40.6)	10.6 (2.2;19.7)	0.9 (0.1;6.7)	0.3 (0;0.7)	381.1 (377.9;395.2)	27.8 (18.5;34.7)
p value[b]		0.06	0.22	0.2	0.77	0.61	0.92
Body Mass Index							
<18.5 kg/m²	10	29.1 (10.4;43.7)	18.2 (7.7;32.3)	0.1 (0.1;4.7)	0.3 (-0.2;0.7)	400.8 (377.4;428.9)	23.1 (17.6;34.7)
18.5-25 kg/m²	69	25 (10.7;56.4)	17.4 (7.7;38.5)	1.1 (0.1;12.4)	0.2 (-0.5;0.8)	406.1 (369;427.2)	24.1 (15.7;35.2)
≥25 kg/m²	16	24.8 (10.4;93.4)	8.3 (2.7;47.3)	0.9 (0.1;6.8)	0.3 (-0.1;0.9)	379.1 (351.9;402.2)	30.6 (18.5;33.8)
p value[b]		0.97	0.76	0.97	0.73	0.16	0.81
Post-secondary school							
2 years or less	50	34.8 (13.4;72.7)	20.9 (6.4;46.3)	3.4 (0.1;16.4)	0.2 (-0.3;0.8)	388.8 (358.3;423.5)	25.9 (16.7;35.2)
≥ 3 years	44	16.6 (9.2;53.6)	11.8 (7.1;32.8)	0.1 (0;4.2)	0.3 (-0.5;0.7)	406.3 (381.8;427.2)	22.2 (14.8;35.2)
Missing value	1						

p value[b]		0.12	0.26	0.07	0.81	0.21	0.52
Occupation at inclusion							
no	8	17.3 (6.6;31.2)	17.4 (5.8;34)	0.1 (0;1.5)	17.3 (6.6;31.2)	17.4 (5.8;34)	0.1 (0;1.5)
yes	87	25.8 (11.1;68.3)	16.8 (6.8;36)	1 (0.1;12.8)	25.8 (11.1;68.3)	16.8 (6.8;36)	1 (0.1;12.8)
Smoker before inclusion							
no	62	25 (9.1;66.2)	16 (5.1;35.3)	0.7 (0;9.1)	0.3 (-0.2;0.8)	403.8 (362;428.9)	27.8 (16.7;33.3)
yes	33	27.1 (14;62)	18.5 (7.2;36.1)	2.8 (0.1;16.2)	0 (-0.6;0.8)	399 (379.2;422.9)	23.1 (13;40.7)
p value[b]		0.54	0.74	0.56	0.47	0.68	0.84
High blood pressure							
no	92	25 (10.4;63.3)	16.9 (6.6;34.4)	0.8 (0.1;9.8)	0.3 (-0.4;0.8)	401.2 (368.3;428.1)	25.9 (16.7;35.2)
yes	3	48.4 (34.3;1706.4)	35.9 (25.8;44.6)	5.2 (3;363.9)	1.3 (-0.1;1.4)	374.3 (368.2;392.8)	13 (10.2;32.4)
Diabetes							
no	94	25 (10.5;60.6)	16.9 (6.7;35.5)	0.8 (0.1;9.1)	0.3 (-0.4;0.8)	399 (367.2;426.4)	25.9 (16.7;35.2)
Missing value	1						
Fruit and vegetable consumption							
<3 portions/day	78	22.5 (10.3;60.6)	16.6 (6.4;33.7)	0.4 (0.1;9.1)	0.3 (-0.2;0.8)	399 (369.7;429.3)	24.1 (16.2;33.3)
≥3 portions/day	17	46.1 (14.8;67.1)	20.4 (11.2;53.4)	3.7 (0.2;16.2)	-0.1 (-0.7;0.4)	408.3 (366.1;423.5)	33.3 (20.4;46.3)
p value[b]		0.14	0.21	0.22	0.12	0.92	0.17
Fish consumption							
< twice a month	62	24.5 (7.5;49.3)	16 (3.7;33)	0.1 (0;7.3)	0.2 (-0.4;0.9)	397.1 (372.5;429.3)	25.9 (16.7;33.8)
≥ twice a month	33	31.1 (13.7;90.2)	17.5 (9.3;60.9)	3.7 (0.1;16.3)	0.3 (-0.3;0.7)	408.3 (366.1;424.9)	24.1 (14.8;35.2)

p value[b]		0.1	0.14	0.01	0.92	0.59	0.83
Newborn characteristics							
Sex							
Boys	41	31.1 (11.6;76)	20.2 (9.9;48.5)	1 (0.1;9.4)	0 (-0.6;0.7)	407 (378.3;428.3)	26.9 (13;36.1)
Girls	54	15.3 (10.5;51.8)	12.2 (4.3;33)	0.6 (0;10.4)	0.4 (-0.1;0.8)	392.6 (358.3;423.5)	24.1 (16.7;33.3)
p value[b]		0.11	0.13	0.51	0.09	0.12	0.89
Gestational age							
≤39 weeks of amenorrhea	44	24.1 (9.7;67.7)	17.2 (6.2;40.7)	0.2 (0.1;6.7)	0.4 (-0.6;0.8)	399.6 (366.1;424.2)	24.1 (14.8;33.3)
>39 weeks of amenorrhea	51	27.1 (11.1;56)	16.8 (6.9;34.8)	2 (0.1;16.2)	0.1 (-0.2;0.7)	400.6 (374.9;428.1)	25.9 (16.7;35.2)
Birth weight							
<3000 g	12	23.9 (5.3;53.9)	22.6 (3.3;38.6)	0.1 (0.1;0.1)	0.1 (-0.6;0.8)	397.7 (372.2;416.8)	25 (21.8;33.8)
≥ 3000 g	83	25 (11.1;67.9)	16.8 (6.8;36)	1.7 (0.1;12.8)	0.3 (-0.3;0.8)	399.6 (367.6;428.9)	25.9 (13;35.2)
Breastfeeding							
≤ 3 months	54	40.4 (13.4;74)	24.8 (7;46.3)	0.9 (0.1;15)	0.3 (-0.4;0.8)	395.8 (360.4;422.7)	29.6 (18.5;37)
> 3 months	41	15.2 (10.2;40.9)	12.1 (6.8;21.4)	0.8 (0.1;7.9)	0.3 (-0.2;0.8)	407 (379.5;436.3)	22.2 (13;31.5)
p value[b]		0.04	0.09	0.7	0.96	0.08	0.03
Child characteristics (at follow-up)							
Parity							
single child	16	23 (13.5;51.3)	18.9 (9.2;32.9)	0.7 (0.1;6)	0.4 (-0.8;0.8)	399 (369.9;424)	30.6 (17.6;35.6)
1 sibling	43	25 (10.3;58.8)	17.2 (6.6;33.7)	1.1 (0.1;8.6)	0.3 (-0.3;0.8)	402.8 (377.2;422.5)	22.2 (16.7;33.3)
≥ 2 siblings	36	25.9 (9.8;70.5)	16.6 (6.3;47.6)	0.5 (0;14.1)	0.2 (-0.2;0.6)	395.3 (366.1;436.4)	28.7 (16.2;36.6)

p value[b]		1	0.87	0.79	0.64	1	0.32
Age							
10 years old	66	25.8 (10.6;64.4)	16.6 (4.3;35.3)	0.5 (0.1;15.5)	0.1 (-0.5;0.6)	409.5 (377.3;434.1)	28.7 (16.2;35.2)
11 years old	29	24 (10.7;62)	17.8 (9.9;48.5)	1.1 (0.1;5.6)	0.7 (-0.1;1.2)	381.4 (360.4;406.3)	22.2 (16.7;31.5)
Educational level							
elementary school	69	27.8 (12.5;73.8)	17.2 (6.8;35.9)	0.8 (0.1;13.3)	0.2 (-0.3;0.7)	404.9 (377.3;425.2)	27.8 (16.7;35.2)
junior high school	26	14.1 (7.5;51.8)	13.6 (4.1;37.3)	0.9 (0.1;5.5)	0.5 (-0.5;1.1)	387 (363;424.9)	20.4 (13;35.2)
p value[b]		0.12	0.6	0.82	0.43	0.54	0.23
Lateralization							
right-hander	83	27.1 (9.8;71.6)	16.3 (5.5;40.6)	1.1 (0.1;12.8)	0.2 (-0.5;0.8)	402.8 (366.9;427.2)	25.9 (15.7;35.2)
left-hander	12	20.4 (14.2;26.9)	18.7 (11.6;28.8)	0.1 (0;0.5)	0.5 (-0.2;0.7)	389.6 (378.1;424.3)	22.2 (18.5;33.8)
p value[b]		0.65	0.74	0.03	0.66	0.78	0.84

Note: DAP: Dialkylphosphate, DE: diethylphosphate, DM: dimethylphosphate

[a]: standardized and imputed OP metabolites levels

[b]: Kruskal-Wallis test for repartition differences between OP metabolites levels or inhibition indicators by characteristic.

Table S. 4: BOLD responses related to motor inhibition demand perception (No-Go vs Go contrast) (n=92).

Structure	Hemisphere	Extent (in voxels)	X	Y	Z
Anterior cingular region/SMA	L/R	3686	4	36	20
Inferior/superior parietal region	R	1948	44	-44	56
	L	164	-46	-40	48
	L	17	-62	-52	36
	L	10	-66	-18	24
Posterior cerebellum	L	918	-40	-52	-44
	L	20	-14	-56	-52
	R	13	40	-48	-32
	R	7	22	-62	-52
Inferior frontal region	R	612	46	18	-8
	R	14	22	42	-12
	L	387	-42	14	0
Middle frontal region	L	553	-36	42	28
Middle temporal region	R	308	58	-28	-4
Caudate nucleus	R	77	8	-2	8
	L	29	-8	0	12
Occipital region	L	29	-20	-100	12
	R	6	16	-102	4

Cluster-wise significance (FWER corrected p=0.05) with a FWER-corrected one-sided cluster-defining threshold of p=0.05.

Table S. 5: BOLD responses related to successful motor inhibition (Successful No-Go vs Successful Go contrast) (n=92).

Structure	Hemisphere	Extent (in voxels)	X	Y	Z
Inferior/superior parietal region	R	2659	44	-44	56
	L	238	-40	-40	48
	L	17	-58	-18	8
	L	7	-68	-20	24
Posterior cerebellum	L	1610	-40	-60	32
	R	112	16	-56	-48
	R	61	40	-56	-28

Middle frontal region	R	858	36	50	20
	R	471	28	0	60
	R	9	36	62	4
Putamen	R	358	20	12	4
	L	251	-24	6	-4
Anterior cingular region/SMA	L/R	296	2	6	48
Occipital region	L	227	-14	-98	16
	L	100	-42	-84	4
	R	107	16	-102	4
Inferior frontal region	L	201	-30	36	20
	L	35	-38	12	4

Cluster-wise significance (FWER corrected p=0.05) with a FWER-corrected one-sided cluster-defining threshold of p=0.05.

Table S. 6: Associations between OP metabolites levels in maternal urine during pregnancy and performance indicators for the Go/No-Go task without statistical outliers (n =91).

	Score		**Response latency**		**Commission rate**	
	β	95% CI	β	95% CI	β	95% CI
DAP (moderate vs. low levels)	0.12	[-0.41 ; 0.66]	0.03	[-0.03 ; 0.09]	-6.55	[-12.85 ; -0.26]
DAP (high vs. low levels)	0.29	[-0.25 ; 0.83]	-0.02	[-0.09 ; 0.04]	-2.3	[-8.73 ; 4.12]
DM (moderate vs. low levels)	0.22	[-0.3 ; 0.75]	0.02	[-0.05 ; 0.08]	-5.45	[-11.69 ; 0.78]
DM (high vs. low levels)	0.34	[-0.2 ; 0.88]	-0.05	[-0.12 ; 0.01]	-0.06	[-6.5 ; 6.38]
DE (moderate vs. low levels)	-0.13	[-0.66 ; 0.41]	0.02	[-0.04 ; 0.09]	0.57	[-5.86 ; 7]
DE (high vs. low levels)	-0.19	[-0.71 ; 0.32]	0.03	[-0.03 ; 0.09]	0.74	[-5.48 ; 6.96]

Table S. 7: Associations between OP metabolites levels in maternal urine during pregnancy and performance indicators for the Go/No-Go task of their 10- to 12-year-old children without standardization on sampling conditions (n =93).

	Score	**Response latency**	**Commission rate**

	β	95% CI	β	95% CI	β	95% CI
DAP (moderate vs. low levels)	0.59	[-0.22 ; 1.4]	-0.02	[-0.11 ; 0.06]	-3.46	[-9.69 ; 2.78]
DAP (high vs. low levels)	0.5	[-0.35 ; 1.36]	-0.05	[-0.13 ; 0.04]	-2.45	[-9.03 ; 4.13]
DM (moderate vs. low levels)	0.74	[-0.08 ; 1.56]	-0.06	[-0.14 ; 0.02]	-4.06	[-10.39 ; 2.28]
DM (high vs. low levels)	0.4	[-0.4 ; 1.21]	-0.07	[-0.15 ; 0.02]	-0.86	[-7.09 ; 5.37]
DE (moderate vs. low levels)	-0.44	[-1.24 ; 0.36]	0.03	[-0.05 ; 0.11]	2.72	[-3.4 ; 8.84]
DE (high vs. low levels)	-0.51	[-1.31 ; 0.29]	0.08	[0 ; 0.16]	-1.31	[-7.47 ; 4.86]

Table S. 8: Associations stratified by sex between dialkylphosphate levels in maternal urines during pregnancy and activation intensity related to inhibition demand during the Go/No-Go task of their 10-12 years old children (PELAGIE cohort. 2002-2017) (FWER correction at p<0.05).

	Levels	N	Inhibition network (frontal or cingular regions)		Other brain regions		
			β (95% CI)	ext.	β (95% CI)	Region	ext.
	DAP						
Boys	*Low*	10	Ref.		Ref.		
	Moder	11					
	High	17			1.4 (0.7 ; 2.1)	Right hippocampus	346
					1.6 (0.7 ; 2.4)	Left hippocampus	1791
Girls	*Low*	21	Ref.		Ref.		
	Moder	19					
	High	14					
	DM						
Boys	*Low*	16	Ref.		Ref.		
	Moder	11					
	High	11			2.9 (0.4 ; 5.3)	Right occipital	299
					1.5 (0.6 ; 2.4)	Left insula	2556
Girls	*Low*	24	Ref.		Ref.		
	Moder	14	-3.2 (-4.9 ; -1.5)[a]	515			
	High	16					
	DE						

Boys	*Low*	11	Ref.	Ref.		
	Moder	12		-1.7 (-2.5 ; 0.9)	Cerebellum	1556
	High	15				
Girls	*Low*	21	Ref.	Ref.		
	Moder	18		-1.3 (-1.7 ; 1.0)	Left precuneus	539
	High	15				

Note: DAP: Dialkylphosphate, DE: diethylphosphate, DM: dimethylphosphate, Low/Moder/High: low, moderate or high OP metabolite levels

[a]: Medial superior frontal

β (95% IC): estimated coefficient [95% confidence interval]. Empty cell: no statistically significant difference reported in this region.

All estimates are from mixed-effect generalized linear regression models adjusted for maternal fruit/legume consumption (less than 3 parts a day vs at least 3 part a day) and breastfeeding duration (less than 3 months vs more than 3 months).

Table S. 9: Associations stratified by sex between dialkylphosphate levels in maternal urines during pregnancy and activation intensity related to successful inhibition during the Go/No-Go task of their 10-12 years old children (PELAGIE cohort. 2002-2017) (FWER correction at p<0.05).

	Levels	N	Inhibition network (frontal or cingular regions)		Other brain regions		
			β (95% CI)	ext.	β (95% CI)	Region	ext.
	DAP						
Boys	*Low*	10	Ref.		Ref.		
	Moder	10					
	High	17	-1.9 (-2.5 ; 1.2)[a]	369	1.4 (0.9 ; 1.9)	Left occipital	921
Girls	*Low*	21	Ref.		Ref.		
	Moder	18					
	High	14					
	DM						
Boys	*Low*	15	Ref.		Ref.		
	Moder	11					
	High	11	-2.0 (-2.8 ; -1.2)[a]	529	1.4 (1.0 ; 1.8)	Left hippocampus	416
Girls	*Low*	23	Ref.		Ref.		
	Moder	14					
	High	16					

	DE						
Boys	*Low*	11	Ref.		Ref.		
	Moder	12	-2.6 (-3.8 ; -1.5)[b]	556	-1.8 (-2.3 ; -1.2)	Right cerebellum	636
			-3.2 (-4.5 ; -1.8)[c]	380			
	High	14					
Girls	*Low*	21	Ref.		Ref.		
	Moder	18					
	High	14					

Note: DAP: Dialkylphosphate, DE: diethylphosphate, DM: dimethylphosphate, Low/Moder/High: low, moderate or high OP metabolite levels

[a]: Medial orbital frontal

[b]: Left middle frontal

[c]: Right middle frontal

β (95% IC): estimated coefficient [95% confidence interval]. Empty cell: no statistically significant difference reported in this region.

All estimates are from mixed-effect generalized linear regression models adjusted for maternal fruit/legume consumption (less than 3 parts a day vs at least 3 part a day) and breastfeeding duration (less than 3 months vs more than 3 months).

Figure S. I: Probability vs urinary OP metabolites concentrations before and after standardization (n=95).

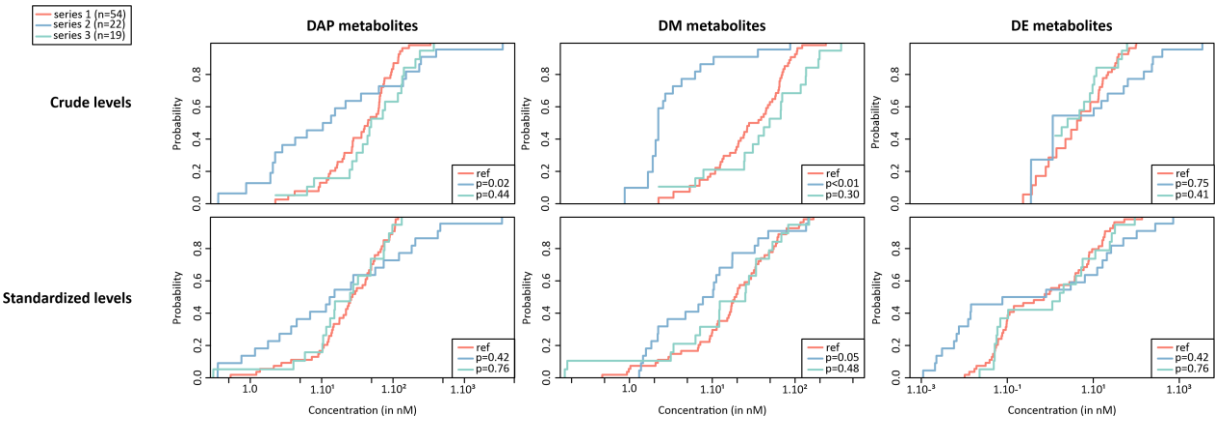
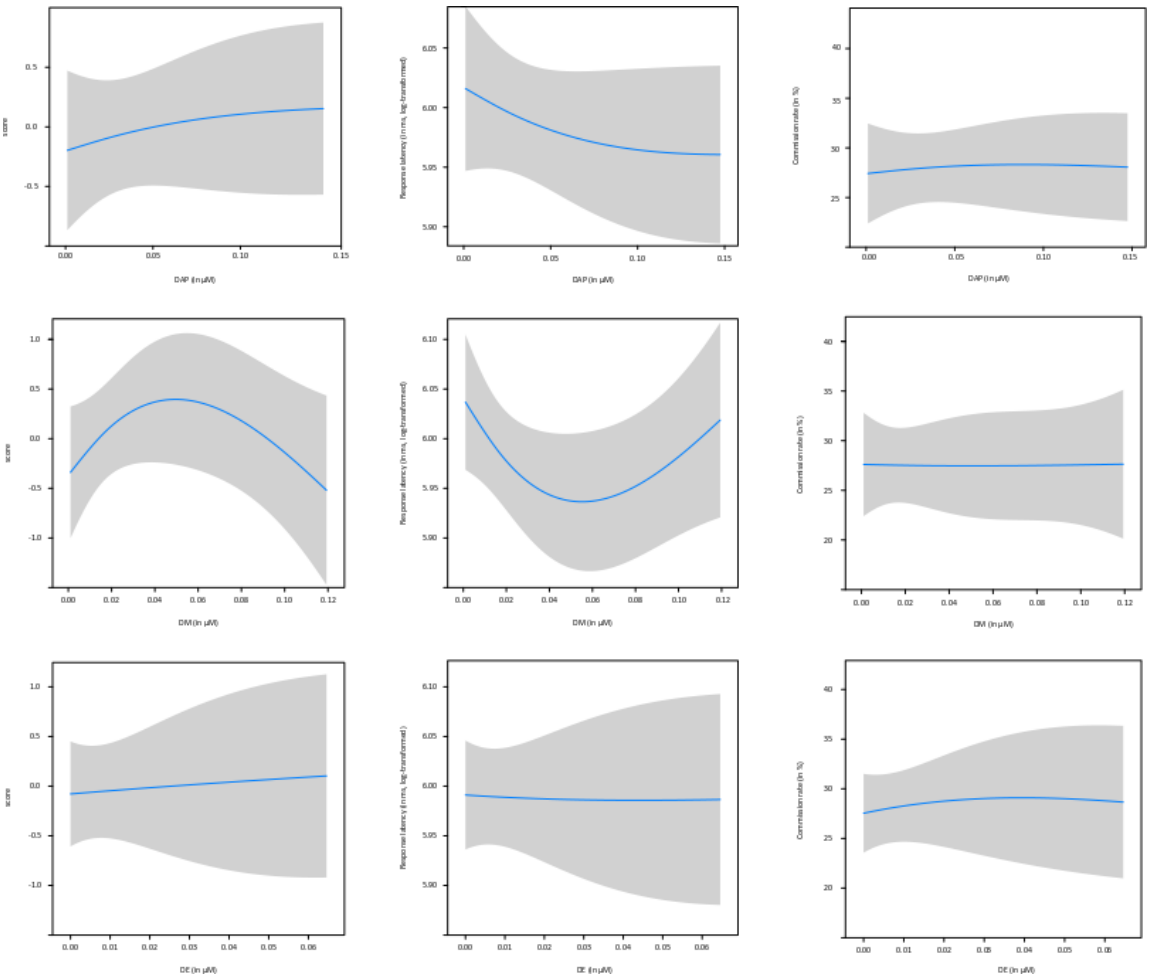


Figure S. II: Dose-response curves for associations between OP metabolites (in µM), reaction time (log-scale, in ms) and performance score.



Restricted cubic splines (continuous line) and 95% confidence intervals (gray areas) adjusted for maternal fruit/legume consumption (less than 3 parts a day vs at least 3 part a day) and breastfeeding duration (less than 3 months vs more than 3 months).

PART IV

CONCLUSION

The vulnerability of both the structure and function of the brain to its environment during developmental times is well known in the literature. Previous studies have reported a possible neurotoxicity of glycol ethers and organophosphate insecticides after exposure during pregnancy. These effects are observed at levels of exposure similar to those observed in the general population. Some cognitive and behavioral alterations observed (i.e. learning disabilities, attention troubles and impulsivity) suggest a possible impairment of executive functions, in particular inhibitory control.
Inhibition during childhood is a strong predictor of outcomes in adulthood, such as academic performance or tendency to risky behaviors. Based on these findings, better understanding the effects of exposure to neurotoxicants on the inhibition function appears as a key issue of public health.
Therefore, this thesis aimed at investigating the effects of prenatal exposure to glycol ethers and organophosphate insecticides on the child's inhibitory control evaluated by neuropsychological tests and fMRI.
This work is based on the follow-up of the population-based PELAGIE cohort. The prospective design of this mother-child cohort allowed to measure with biomarkers the exposure to neurotoxicants during a vulnerable period. The Go/No-Go task and fMRI were used to assess the inhibitory control and to investigate neural underpinnings of this executive function in children aged 10-12 years.

1.1. Synthesis of the main findings

In the first study about brain activation during a Go/No-Go task, we aimed at describing BOLD responses of 10-12 years old children recruited in the general population and assessing correlation between brain activation and neuropsychological tests. Based on previous work by Mostofsky *et al.*, the visual Go/No-Go task presented here has a simple design in order to limit cognitive demand other than inhibition of a prepotent response[1]. It has been used in adults[1], ADHD-children at age 8-13 years[2] but never in a large group of neurotypical children. Attention scores were based on attention subtests of the NEPSY battery and inhibition scores were built from performances at the Go/No-Go task. Children of this age are able to perform the 10 minute-task in a MRI scanner without significant fatigue and with a good observance. By the use of contrasts during statistical modeling, we reported brain activations in bilateral frontal regions, including lateral inferior frontal areas, anterior cingular cortex and SMA, in agreement with previous studies on motor inhibition. These findings confirmed our hypotheses to study motor inhibition in 10-12 year-old children from general population as ours. In addition, we reported few correlations

1. Mostofsky et al., "fMRI evidence that the neural basis of response inhibition is task-dependent"
2. Suskauer et al., "Functional Magnetic Resonance Imaging Evidence for Abnormalities in Response Selection in Attention Deficit Hyperactivity Disorder".

between brain activities during the tasks and neuropsychological scores of attention or inhibition. We found some correlations between BOLD responses in cingular or inferior frontal regions and behavioral scores (attention and commission rate). This study suggests that BOLD intensity is not the unique predictor of performances at the Go/No-Go task, and that neuropsychological tests and fMRI are complementary techniques.

About prenatal exposure to GE, we reported a poorer performance in children with high levels of BAA or increasing levels of EAA. We found no association between maternal urinary concentrations of metabolites and neural activity related to inhibition demand in regions involved in the motor inhibition network. We reported associations between increased child brain activation in the left cuneus and moderate levels of EAA and increased activity in the right precuneus and moderate MAA concentrations in maternal urine, but not with the highest levels of exposure. We observed associations between decreased child brain activation related to successful inhibition in frontal regions, and high levels of EEAA and between increased activity and moderate PhAA concentrations in maternal urine. Few studies have investigated the possible toxicity of GE on developing brain. In the PELAGIE cohort, Beranger *et al.* have reported associations between PhAA and verbal comprehension scores and EAA and visuospatial performance[3]. An animal study on gestational rats showed learning and neuromuscular disabilities and neurochemical alterations after administration of EGEE and EGME (respectively metabolized into EAA and MAA) at doses showing no effects on parents[4]. Therefore, some of our findings were unexpected regarding existing literature and there is no consistency between performance indicators and cerebral activity in our results. Our study does not enable us to conclude to a significant neurotoxicity of GE on developing brain.

Investigating the potential neurodevelopmental toxicity of OP, we found no major alteration of children performance, only a slight and non-significant decrease of composite performance score with moderate levels of DE. For moderate levels of DAP, a decreased commission rate suggests better performances. There were no differential brain activations related to inhibition demand, but decreased cerebral activities related to successful inhibition in bilateral superior frontal regions and left inferior frontal regions, with DE and DM respectively. An animal study on dams reported reduced neuron and glia count in anterior cingulate, prelimbic, and infralimbic areas of medial prefrontal cortex after prenatal exposure to chlorpyrifos at levels showing no pathological changes in medial prefrontal cortex[5]. So far, two published epidemiological studies have used neuroimaging techniques to evaluate effects on the brain

3. Béranger et al., “Prenatal Exposure to Glycol Ethers and Neurocognitive Abilities in 6-Year-Old Children”.

4. Nelson and Brightwell, “Behavioral teratology of ethylene glycol monomethyl and monoethyl ethers.”

5. Chen et al., “Mother gestational exposure to organophosphorus pesticide induces neuron and glia loss in daughter adult brain”.

of prenatal exposure to OP. While they were not investigating a specific brain function, Rauh *et al.* reported that 7 year-old children with higher prenatal exposure to chlorpyrifos presented frontal cortical thinning[6]. In a pilot study using fNIRS technique on CHAMACOS adolescents, Sagiv *et al.* have reported a negative association between prenatal exposure to OP and brain activation in the prefrontal cortex (bilaterally) during a cognitive flexibility task, but not during the Go/No-Go task[7]. Our results suggest that pattern of activation associated with inhibitory control may be altered after prenatal exposure to OP and that frontal cortex may be a specific target of OP, in line with the existing literature.

1.2. Methodological considerations

1.2.1. Use of biomarkers

In the present work, both glycol ethers and organophosphate exposure have been assessed with the use of maternal urinary biomarkers.

There are other methods to indirectly measure levels of exposure. Geographic information system (GIS) allowed to evaluate exposure from a geographical context (e.g. proximity to a contaminant source, models of air pollution), even retrospectively, but gives few or no information about specific exposure of an individual. Job-exposure matrix (JEM) is an objective tool to assess exposure in occupational contexts but overlook other sources of exposures. Self-reported questionnaires are inexpensive to assess exposure to contaminants but may be biased by the subjectivity of the respondent.

Mother-child cohort, with a recruitment period in pre-conception or early pregnancy allowed measuring prospectively biomarkers of exposure. These biomarkers provide information about the internal dose of contaminants of an individual and aggregate all sources of exposure. Since pregnant women may be exposed to GE and OP via different conditions, occupational and domestic uses for GE, residential proximity, diet and domestic uses for OP, it seems relevant to use biomarkers to represent the entire exposure.

Using biomarkers, the feasibility and cost of sample collection are important parameters. Collection of maternal saliva, hair or urine can be easily completed by a study participant during pregnancy, while collection of blood requires trained medical personnel and cord blood can be collected only at birth. Moreover, studies have reported that participation rate was higher for collection of non-invasive specimens[8]. Nevertheless, the use of biomarkers also has its limits. First, maternal urine samples reflect doses of contaminants excreted from the mother's organism and not

6. V. A. Rauh, Perera, et al., "Brain anomalies in children exposed prenatally to a common organophosphate pesticide".

7. Sagiv et al., "Prenatal exposure to organophosphate pesticides and functional neuroimaging in adolescents living in proximity to pesticide application".

8. Tworoger and S. E. Hankinson, "Use of biomarkers in epidemiologic studies".

fetal brain levels, which should be of better interest. Then, even if we used "specific" biomarkers of exposure, there is a risk of residual confounding with other chemicals or sources. EGPhE is frequently associated with parabens as preservatives[9] and this uncontrolled co-exposure may bias association estimates[10]. In the same way, OP are known to have a short half-life, from a few days to several months according to environmental conditions. Therefore, they are found in fruits and vegetables in their metabolized form and measures of DAP in urines may over-estimate the true levels of exposure to OP compounds.

An alternative to urinary DAP levels is to measure PON1 or AChE activity in the blood. These enzymes are specific targets of OP and a modification of their activities should be a biomarker of effects.

The best way to model and understand how a xenobiotic is affected (toxicokinetic) and affects (toxicodynamic) an organism is the use of pharmacokinetic/pharmacodynamic (PK/PD) models, but they are complex and not developed for all the xenobiotics. Moreover, pharmacokinetics parameters can be modified during pregnancy, requiring adapted models.

Sampling conditions are other parameters that can influence biomarker concentrations. Urine samples are usually stored in freezers, though storage temperatures vary from -20°C to -196°C. These temperatures are expected to prevent sample and biomarker degradation, but the long (up to 10 years) and different duration of storage found in our studies may increase the variability and bias exposure estimate. Standardizing sampling conditions as much as possible (e.g. date of sample collection, time of day of sample collection) and recording sampling parameter (e.g. problems with processing, time until freezing, length of storage time, or storage temperature, among others) seem of particular interest for limiting heterogeneity of samples and its influence on further associations studies[11]. More and more studies also advocate for the repeated collection of urinary samples in order to take into account time-variability of the exposure[12].

Indeed, the major limitation of this work is the use of only one urine sample collected in early pregnancy (first morning void, before 19 WG). Given the short plasmatic half-life of chemicals of interest, it is unlikely that we are representative of the exposure of the entire pregnancy. There are correlations between concentrations of GE measured in the PELAGIE cohort and occupational exposure during pregnancy

9. Agence nationale de sécurité du médicament et des produits de santé (ANSM), “Evaluation du risque lie a l’utilisation du phenoxyethanol dans les produits cosmetiques”.

10. Béranger et al., “Prenatal Exposure to Glycol Ethers and Neurocognitive Abilities in 6-Year-Old Children”.

11. Mortamais et al., “Correcting for the influence of sampling conditions on biomarkers of exposure to phenols and phthalates”.

12. Vernet et al., “Within-Day, Between-Day, and Between-Week Variability of Urinary Concentrations of Phenol Biomarkers in Pregnant Women”.

assessed by JEM[13]. We could hypothesize that urinary measures are reflecting frequent and repeated exposure but it could be modified during the pregnancy by protection measures at work and maternity leaving for GE or seasonal exposure (e.g. changes of diet or of uses of insecticides) for OP.
According the National Research Council Committee, DAP are classified as group II biomarkers[14]. It means that sampling and methods are reproducible and level of biomarkers informs about the internal dose of exposure. However, the relationship between the external dose and the biomarker is unknown, in animals and humans. For GE, the analytical method (GC-MS) used here seems to be sensitive enough for the evaluation of glycol ethers exposures at low levels in the general population[15].
We could expect that we are able to reflect exposure during the first weeks of fetal life, a key period of brain vulnerability with neurogenesis and neural migration. Other periods during pregnancy and childhood are also sensitive, with the formation of synapses and myelination processes and we were unable to evaluate the possible effects of exposure to GE and OP at these times.

1.2.2. The Go/No-Go paradigm

Neurodevelopmental outcomes investigated here are not diagnosed troubles or pathologies, such as ADHD, but a more subtle phenotype of inhibitory control in children. It allowed investigating fine, subclinical effects of environmental contaminants. We used a neuropsychological test, the Go/No-Go task. Compared to self-report questionnaires, tests are more objective because based on observable data, not biased by the subject's self-perception then less prone to measure and misclassification errors, limiting the risk of bias in association studies and closer to biological models of inhibition[16].
The Go/No-Go paradigm and the Stop-Signal Task are usually used to measure the ability to inhibit a prepotent response. The main difference between Stop-Signal Task and Go/No-Go paradigms is the timing of presentation of the stop, or no-go cue, relative to the go stimulus. In Go/No-Go tasks the no-go signal is presented unexpectedly in place of the go signal, whereas in the Stop-Signal Task the go cue always precedes the stop-signal. This means that the first one measures the inhibition of a planned response ("action restraint") and the latter the inhibition of an already started action ("action cancellation")[17].
Nevertheless, the metrics of neuropsychological tests are not validated. Commis-

13. Garlantézec, Multigner, et al., "Urinary biomarkers of exposure to glycol ethers and chlorinated solvents during pregnancy".
14. Sudakin and Stone, "Dialkyl phosphates as biomarkers of organophosphates".
15. Labat et al., "Dosage des métabolites urinaires des éthers de glycol par chromatographie en phase gazeuse couplée à la spectrométrie de masse".
16. Bari and Robbins, "Inhibition and impulsivity".
17. Chambers et al., "Insights into the neural basis of response inhibition from cognitive and clinical neuroscience"

sion errors (i.e. incorrect answers to no-go cues) are expected to be an indicator of the inability of the child to restrain its gesture. However, this indicator overlooks potential compensatory strategies developed by the child, such as slowing down its reaction. Therefore, it seemed relevant to build a composite score, as suggested by Collignon *et al.*, to obtain a general index of performance including speed and accuracy[18].

Indeed, the Go/No-Go task does not measure pure forms of inhibition, but requires ancillary processes to operate successful response inhibition. This lack of specificity is not necessarily detrimental to the scientific value of the task. In everyday life, adaptive inhibition requires a multitude of interrelated processes such as the monitoring of behavior, sustained attention, conflict detection and others, before the inhibition of an action or the adjustment of behavior according to the new goal-oriented plans are put in place[17].

Question remains about how well a neuropsychological task is the reflect of an executive function in real life. Moreover, the task is adapted to be performed in a MRI scanner and the fact of remaining still, lying down, in a noisy environment gives necessary different conditions[19].

1.2.3. fMRI statistical modeling

The use of neuroimaging to evaluate brain structure and function is quite recent in the field of environmental epidemiology and there is to date few existing studies using task-based fMRI in association with prenatal exposure to environmental contaminants.

Some methodological choices to model BOLD signal were based on existing imaging literature to provide comprehensive results and may have influenced the findings, at least their statistical power.

First, our population is based on youth from the French general population. It is likely that the shape of their brain is not identical to an adult's one. We have chosen to create our own template using diffeomorphic image registration (also known as DARTEL)[20]. Other methods exist, we could have used a template from adults, considering our population old enough, or a template based on children of similar age. Nevertheless, these templates based on a different population are expected to be less adapted and precise[20].

Head motion can artificially change the intensity of the BOLD signal[21]. Authors usually recommend excluding subjects or sequences of the acquisition with excessive head motion but there is no consensus on a threshold. Our outcome of interest be-

18. Collignon et al., "Women process multisensory emotion expressions more efficiently than men".
19. Bennett et al., "Behavior, neuropsychology and fMRI".
20. Ashburner, "A fast diffeomorphic image registration algorithm"
21. Power et al., "Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion".

ing inhibitory control, we could expect that children having troubles to inhibit would have more difficulties to remain still. To not bias our population sample, we only excluded children with head motion exceeding a plane voxel (2 mm) and add motion parameters as regressors to improve the accuracy of our model. Then we did not correct the data for physiological motion.

BOLD responses were modeled using a generalized linear model (GLM) with the canonical HR function and its temporal derivative. The canonical function of SPM provides one estimate to describe the intensity of the answer[22]. There are other temporal basis functions, such as the finite impulse response, which attempt to better describe the shape of the individual HR locally but whose results are more complex and less comprehensible. There are model strategies other than GLM, such as psychophysiological interactions or dynamic causal modeling. The first one estimates interactions and relative contributions of factors (e.g. presentation of go and no-go cues), the second one is a method for making inferences about neural processes that underlie measured time series, considering a neuronal system model. Then it should be noted that other tools (e.g. FSL, AFNI) are available for modeling data from fMRI acquisition and are based on slightly different modeling approaches.

For our association studies, we considered two contrasts of interest: “No-Go vs Go”, activation amplitudes higher for inhibition than motor action, regardless of child’s answers and “Successful No-Go vs successful Go” when children were able to inhibit, specific of successful inhibition. These contrasts are different approaches of our question of interest (i.e. does exposure modify brain activity during inhibition?) and both built not to be specific to the pure inhibition (i.e. mechanism that actively suppresses the motor command) and probably include ancillary processes to inhibition[23]. Studies investigating the effects of environmental contaminants on brain function are sparse, therefore, it seemed relevant to include processes like selective attention or decision making, in the scope of our investigations.

We used a whole brain strategy to investigate possible compensatory brain activations. In addition, we did not have strong *a priori* on brain regions that could be affected by exposure to chemicals. Moreover, there is still no consensus on the regions involved in the inhibition network and it was complex to precisely locate these regions of interest (ROI). Nevertheless, to increase the statistical power of association studies, we could have used ROI analysis and limit the number of voxels.

Last, there are relatively few published studies using the Go/No-Go task during an fMRI sequence, which makes it difficult to interpret the results. Here, we are only investigating the intensity of the HR but other parameters, such as the latency of the peak or its exact localization, may also be involved in the inhibition process.

22. Friston et al., “Event-Related fMRI”.

23. Criaud and Boulinguez, “Have we been asking the right questions when assessing response inhibition in go/no-go tasks with fMRI?”

2.1. Implications in public health and the gap to causal proof

Along with their limits, findings suggest an association between prenatal exposure to environmental contaminants and inhibitory control. However, statistical associations are not formal causal evidences. One way to establish causation is to identify the mechanisms of neurotoxicity. Nevertheless, their diversity and the specific limits of experiments make it difficult to do so. Other steps of this work to approach causal evidence could be to replicate the results, continue long-term research and integrate other environmental factors, both social and chemicals, set aside in this work. Nevertheless, the transfer of knowledge to society from epidemiological studies is already being done and should be more delicate and less comprehensible with neuroimaging.

2.1.1. A diversity of possible mechanisms of neurotoxicity

Modes of actions of environmental factors and contaminants on human neurodevelopment are far from being well understood. A well-known target of OP is AChE but at low concentrations it is likely that non-cholinergic mechanisms are involved, such as alteration of axonal transport or mitochondria metabolism, apoptosis of neurons and glial cells or effects on the metabolism of other neurotransmitters. Neurodevelopmental effects of GE are less investigated but the few existing animal studies have also suggested possible neurochemistry alterations.

The literature suggests several mechanisms to explain neurodevelopmental effects of prenatal exposure to ethanol. The review of Inserm collective expertise reported that even at low levels of exposure, both glial and neuronal metabolisms can be altered. They reported possible effects on neurotransmitters, receptors and gene differentiation and all these effects may lead to behavioral and functional deficits[1].

Tran et Miyake also pointed out epigenetic mechanisms (i.e. molecular alterations in gene expression without changes in DNA sequence) as a possible pathway between exposure to environmental contaminants, such as persistent organic pollutants or heavy metals, and autistic disorders and ADHD[2].

Endocrine mode of action is a potential mechanism of chemicals but it is very unlikely that it is the only one. Schug *et al.* reviewed the potential mechanisms of endocrine disruptor compounds on neurodevelopment. They found as expected mode of actions involving hormone metabolisms (e.g. disruption of thyroid hormone metabolism for PCBs or inhibition of testosterone synthesis for phthalates) but several others were not linked to hormonal system. They reported alteration of neurotransmitter metabolism (production, transport or uptake), neuronal maturation or connectivity,

1. Institut national de la santé et de la recherche (INSERM), *Alcool*.
2. Tran and Miyake, “Neurodevelopmental Disorders and Environmental Toxicants”.

receptor binding or epigenetic modeling[3].

The diversity of possible neurotoxicant mechanisms makes it difficult to precisely understand how exposure to environmental contaminants lead to behavioral or cognitive alterations in children. Moreover, mechanistic studies are usually performed on isolated cells or tissues (i.e. *in-vitro*) or with animal models (i.e. *in-vivo*). *In-vitro* experiments overlook the complexity and inter-relation of biological systems. Animal models can present inconsistencies with humans and make it complex to extrapolate results to humans. Then, mechanistic studies are carried out in very controlled conditions, and sometimes with unrealistic levels of exposure compared to humans. Therefore, interdisciplinary efforts between the neurobiology and environmental science could address how environmental toxicants trigger underlying biological mechanisms, but there are also both needs and opportunities for epidemiology to get closer to causal evidence.

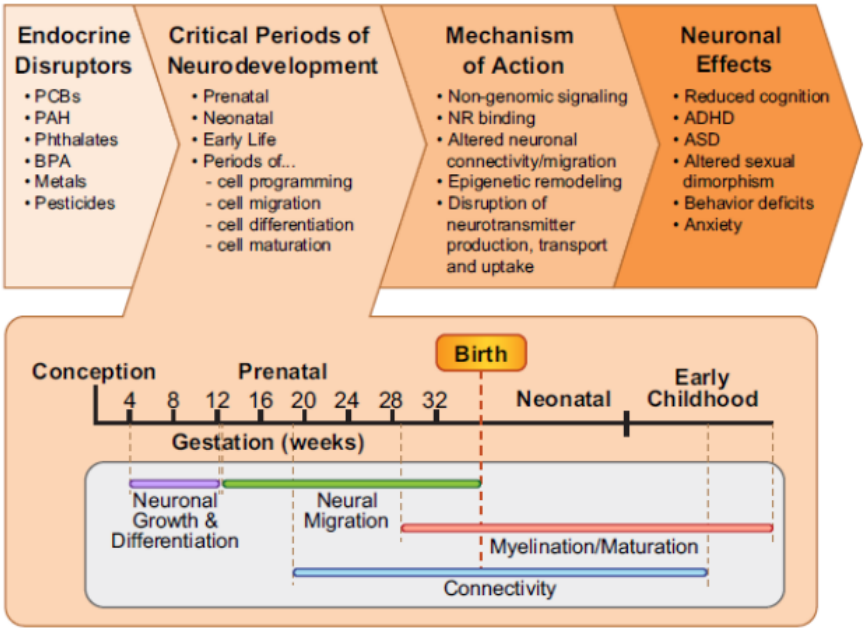


Figure 2.1 – Possible modes of actions of environmental contaminants on brain function[3].

2.1.2. A need to extent to other studies

Prospective mother-child cohorts are powerful to study health effects of early environmental exposures by measuring exposures before assessing outcomes of interest, then limiting the risk of memorization bias that could occur in retrospective or cross-sectional studies. However, attrition is a common threat of the prospective cohort. Here, half of invited families declined to participate. There is a risk of attrition bias on association estimates if the participation is influenced by both the exposure

3. Schug et al., “Elucidating the Links Between Endocrine Disruptors and Neurodevelopment”

level and the outcome of health, i.e. people with poorer health status drop out or, at contrary, over-participation of people with altered health to get an *explanation* of their condition. In this case, data are missing not at random (MNAR) and the loss of information biases estimates. We investigated a sub-clinical outcome and it is unlikely that abilities of inhibition function, unknown to the child and its family, are directly driven their participation to the study. Moreover, we found no socio-economic differences between included and non-included (refusals or exclusion) suggesting that included children are representative of our eligible population.
The PERINE population is a homogeneous group from a highly educated population, with children of same age and restriction on main risk factors of poorer development. Nevertheless, even if statistical models were adjusted on potential confounders, we cannot rule out residual confounding, due to uncontrolled co-exposures or other unmeasured risk factors.
Then, as previously noted, our population is *selected*. Neurotoxicant effects have been estimated for a given population, exposed prenatally to alcohol nor to tobacco and it could be interesting to extend the study to people from disadvantaged contexts to investigate whether or not effects of exposure to GE or OP could be potentiated or reversed in the presence of other risk factors of poorer neurodevelopment.
For example, first epidemiological studies about early exposure to OP were based on deprived population (e.g. Mexican-American agricultural communities in the CHAMACOS cohort). They reported adverse effects of exposure on child's neurodevelopment, but similar cohort in Europe (e.g. PELAGIE in France or Generation R in the Netherlands) found more subtle and inconsistent effects. In addition of the different levels of exposure, authors suggested that the higher socio-economic status observed in the European cohorts may protect against adverse effects of exposure to OP.
Therefore, it seems of great interest to investigate other population in order to reproduce the results of the present study and to reinforce the findings.

2.1.3. A call for long-term research

This study is a continuation of a previous PhD thesis conducted within the PELAGIE cohort and based on the follow-up at age 2 years. By investigating association between maternal occupational exposure to solvents and behavior scores of the 2 year-old children, authors suggested a positive association between prenatal exposure and attention deficit/hyperactivity and aggression scores (deleterious in term of behavior)[4]. The present study, for one part, allowed to test hypotheses based on previous findings (i.e. an alteration of executive functions), to take a closer look into possible altered neural function and investigate whether effects persist in child-

4. Pelé et al., "Occupational solvent exposure during pregnancy and child behaviour at age 2".

hood[5].

Indeed, a previous study in the PELAGIE suggested that associations between solvents and behavior might be attenuated at age 6 years[6]. Authors provide three possible explanations: transient effects, reversibility, or setting up of compensatory mechanisms during childhood.

Functional neuroimaging is an ideal technique to disentangle the hypotheses by studying neural underpinnings of behavioral, cognitive or executive functions and highlighting possible compensatory mechanisms.

Nevertheless, we cannot rule out that several events during childhood, not evaluated here, may enhance or impair inhibition function. Education, sport training, life experiences (both positive and negative) are prone to impact inhibitory control. Moreover, due to the significant plasticity of the brain, inhibition can be learned, trained, and improved, like other cognitive skills[7].

Therefore, long-term researches are of great value for studying how child's environment could affect the neurodevelopment, independently or not of prenatal exposure. By identifying possible mediators of association, it should give more weight to the results of epidemiological studies.

2.1.4. Cocktail of pollutants in everyday-life

Another further research opportunity is the integration of multiple exposures. Here, we focused on two classes of chemical but humans are exposed to hundreds of compounds at the same time. Current metabolomics techniques allow investigating multiple compounds and their effects by measuring simultaneously biomarkers of exposure and effects in biological matrices[8]. Nevertheless, complex techniques are involved in multi-pollutants approaches, from chemical analyses to statistical modeling (e.g. How to take into account the correlation between compounds? Are we looking for the effect of a specific compound or the total effect of the mixture?). Both multiple-exposure and neuroimaging are emerging approaches in the field of environmental epidemiology and it seems difficult to merge them in current studies.

2.1.5. Preventing and promoting environmental health

In environmental health, the precautionary principle applies when there is a suspected risk on health but the hazard or mechanisms are not fully understood.

About GE, several regulation decisions have restricted the use of the most concerning GE in particular to protect pregnant women at work. Decisions were not based on potential neurotoxicity of the GE but rather on their reprotoxic properties. Our study

5. Pelé, "Environnement et développement de l'enfant à 2 ans".
6. Costet et al., "Occupational exposure to organic solvents during pregnancy and childhood behavior".
7. Spierer et al., "Training-induced behavioral and brain plasticity in inhibitory control".
8. Bundy et al., "Environmental metabolomics".

suggests that the levels of exposure in the PELAGIE population are not associated with significant adverse effects on neurodevelopment. Nevertheless, exposure of the general population still seems to be relevant today. In the PELAGIE cohort (2002-2006), GE metabolites were detected in almost all maternal urines. In addition, a recent report from Santé Publique France based on the Esteban study (2014-2016) found at least one metabolite (MAA, MEAA, EAA, EEAA, BAA, PAA, PhAA or 2-MPA) in urines of the whole (adult and child) population, suggesting ubiquitous exposure of the French population, through the use of cosmetics and cleaning products[9].

Several OP insecticides have been banned in France since the last decade (cf. 2.3.4) meanwhile studies investigating long-term effects of exposures are still ongoing.
At the individual level, the reduction of domestic uses of pesticides and increasing the part of organic foods in diets should also be efficient in order to reduce exposure to pesticides[10]. Public policies restricted residue levels in diet, pesticide spraying next to care centers, schools or residential areas should eventually reduce levels of contamination of the population. However, in the field of pesticides (wider than just OP insecticides), some decisions such as the minimal distance between pesticide uses and residence are still not coordinated in 2019. From the ANSES notice in June 2019 suggesting 3 to 10 meters to individual decisions of city mayors to restrict pesticide use beyond 150 meters, there is no consensus on eventual regulation[11].

2.1.6. Subtle alterations in my brain, should I panic?

A "small" functional effect that is still considered to be in the normal range may nonetheless have important consequences for a society, particularly if a large segment of the society is exposed.
It is now well established that a common effect of exposure to developmental neurotoxicants is a small decrement in individual IQ scores. However, a shift in the IQ of the population by 5 points would result in a doubling of the number of individuals with IQ < 70 who would require greater resources, in particular educational and social[12]. The population at risk of subclinical neurotoxicity from industrial chemicals is very large. In the European Union, methylmercury exposure is estimated to an annual economic loss close to €10 billion. In France alone, lead exposure is associated with annual costs that might exceed €20 billion, due to IQ losses[13]. Nevertheless, it is not easy to measure the burden of neurodevelopmental disorders because of

9. Santé publique France, “Imprégnation de la population française par les éthers de glycol. Programme national de biosurveillance, Esteban 2014-2016”.
10. Barrault, “Responsabilité et environnement”; Lu et al., “Organic diets significantly lower children’s dietary exposure to organophosphorus pesticides”.
11. Agence nationale de sécurité sanitaire de l’alimentation, de l’environnement et du travail (ANSES), *Avis de l’Agence nationale de sécurité sanitaire de l’alimentation, de l’environnement et du travail relatif à une demande d’appui scientifique sur les mesures de protection des riverains lors de l’utilisation des produits phytosanitaires*.
12. Rice and Barone, “Critical periods of vulnerability for the developing nervous system”
13. Grandjean and Landrigan, “Neurobehavioural effects of developmental toxicity”.

their co-occurrence, under-diagnosis in certain sociodemographic groups and indirect social and medical costs[14].

Another form of latent consequences after developmental exposure to neurotoxicant agents is the increase of early deficits with maturation to adulthood. As the normal brain ages, there is a decrease in the cell number in certain regions, as well as a decline in neurotransmitter levels and repair mechanisms. The consequences of a potential acceleration of the aging processes after developmental exposure to a neurotoxicant agent are significant. As the large cohort of baby boomers ages, the costs of neurological or psychological dysfunction will be significant, and place additional burdens on the cost and infrastructure of the health care system[12].

Thus, poorer performances may not be considered as pathological but nevertheless reflect brain function alteration. Moreover, adding neurotoxicant exposures sources (e.g. aging, stress, chemicals) to vulnerable populations may trigger cognitive dysfunctions. Increasing costs or incidence of pathologies or reduced IQ are easier to communicate to the society than subclinical effects. The question remains about how communicate in public health on the results of epidemiological studies, moreover when the research question focuses on brain function alterations, which could appear of great concern, but without pathology or even changes of performances, suggesting subtle effects.

2.2. Issues of interdisciplinary researches

In the field to environmental epidemiology, neuroimaging studies investigating effects of prenatal exposure on brain structure and function are sparse but the interest for understanding the neural basis of cognitive or behavioral alterations and the sensitivity of neuroimaging are increasing the body of interdisciplinary studies.

One of the first studies using MRI to investigate associations between prenatal exposure to PCBs, volume of the corpus callosum, and response inhibition in 4.5 year-old children. They reported that splenium size predicted errors of commission, with smaller size associated with more errors of commission and the smaller the splenium, the larger the association between PCBs and errors of commission[15]. Rauh *et al.* found positive association between chlorpyrifos exposure and enlargement of superior temporal, posterior middle temporal, and inferior postcentral gyri bilaterally, and enlarged superior frontal gyrus, gyrus rectus, cuneus, and precuneus along the mesial wall of the right hemisphere. In addition, they reported a statistically significant interaction between exposure × IQ interaction and surface measures of the superior temporal and inferior frontal cortices[16]. More recently, a study found negative

14. Cleaton and Kirby, “Why Do We Find it so Hard to Calculate the Burden of Neurodevelopmental Disorders”.

15. Stewart et al., “Prenatal PCB exposure, the corpus callosum, and response inhibition.”

16. V. A. Rauh, Perera, et al., “Brain anomalies in children exposed prenatally to a common organophosphate pesticide”.

associations between particulate matter levels during fetal life and cerebral cortex in precuneus and rostral middle frontal regions. The reduction of the cortex volume partially mediated the association between exposure and impaired inhibitory control[17]. Both the studies have suggested that alterations of the brain *structure* could serve as mediators in the relation between prenatal exposure and executive and cognitive *function*. Nevertheless, these findings have not yet been confirmed by independent replication.
There are even fewer studies using task based-functional imaging techniques to investigate neural basis of (altered) brain function. Faroese adolescents underwent photic stimulation and a motor task during a fMRI sequence. Increasing exposure to methyl-mercury and PCBs was associated with altered patterns of brain activation during the tasks[18]. In the same way, a California pilot study reported altered brain activation during tasks of executive function (cognitive flexibility and working memory) in children prenatally exposed to OP[19].

Neuroimaging is a tool for environmental epidemiologists to visualize brain structure and function and investigate another angle of neurodevelopment. Due to its complexity, there is a need of expertise of other disciplines, from MR physics to image processing and neuropsychology. These research domains share similar concepts (e.g. statistical modeling, correction for multiple testing) but use different vocabulary or different approaches. In clinical research, MRI is usually used to compare two groups (patient versus control) whereas in epidemiology, there is not always patients. The interest in epidemiological research is to estimate dose-response relationship between exposure and outcome, if any. Epidemiological studies usually include hundreds of people, to satisfy enough statistical power. Due to the cost and technical constrains, it seems difficult to perform fMRI studies at such a large scale. Then, the opportunity and feasibility of research could be limited by a lack of compliance; children and families could be reluctant to undergo MRI for research and not clinical purposes. Nevertheless, the last remarks have to be qualified: in our study, participation rate was around 50%. Moreover, a study from Generation R using MRI to investigate the association between prenatal exposure to air pollution and brain morphology in children included almost 800 school-age children[17]. It suggests that neuroimaging may be acceptable for research purposes in the general population and could be scaled to larger sample size.
There may be an interest of using more portable and less expensive techniques such as fNIRS. In the CHAMACOS study, a pilot study have shown that fNIRS is sensitive enough to discriminate the pattern of brain activation across different levels of

17. Guxens et al., “Air Pollution Exposure During Fetal Life, Brain Morphology, and Cognitive Function in School-Age Children”
18. White et al., “Functional MRI approach to developmental methylmercury and polychlorinated biphenyl neurotoxicity”.
19. Sagiv et al., “Prenatal exposure to organophosphate pesticides and functional neuroimaging in adolescents living in proximity to pesticide application”.

prenatal exposure to environmental contaminants. A strength of this fNIRS study is the administration of six cognitive tasks in a limited period (around 90 minutes) to cover different aspects of cognitive and executive functions. Nevertheless, there are limits in this study: regions of interest have to be determined *a priori* from previous epidemiological studies and are restricted to the cortical surface. Moreover, the indicator of brain activity (variation of the oHb rate) is still indirect and is not easier to interpret than fMRI.

2.3. Perspectives of research

2.3.1. Investigate other cognitive functions

Further steps of the present research should be to investigate other executive functions, such as working memory, which may be altered after prenatal exposure to solvent or pesticides. Moreover, the present study suggests a possible alteration of attention abilities that should be studied with a dedicated task.
There is also an interest to study the impact of subtle effects such as alterations on executive functions, at individual level on scholar or professional future or aging, as well as the consequences for the society.

2.3.2. Take advantage of the multi-modality of neuroimaging

A major strength of MRI is the possibility to assess multi-modal sequences. Microscopic structure from VBM and structural connectivity from diffusion are valuable to interpret the differential brain activities associated with exposure to chemicals. They could highlight potential mechanisms of alteration occurring in the early life (reduction of the cortex or lack of neural fibers) and facilitate the interpretation of the findings from functional studies. Other indicators have been collected for the present study that are beyond the scope of this thesis. Then, neurosciences could also bridge the gap between behavior and cognitive function and their neural basis.

2.3.3. Improve the knowledge about exposure to environmental contaminants

It seems relevant to promote studies with a prospective design for measuring exposure and to look closer at the specific time-period of brain vulnerability. To do this, repeated measures are needed from early pregnancy to childhood.
There is also a need to assess the biological plausibility of the observed effects, by involving other research fields but also by implementing suitable methods in epidemiology.

2.3.4. Monitoring and preventing environmental health

Then, it is essential to pursue the monitoring of the exposures of the general population to understand the sources and the impacts of intervention studies or public

policies on the levels of exposure[20].

20. Santé publique France, “Imprégnation de la population française par les éthers de glycol. Programme national de biosurveillance, Esteban 2014-2016”.

References

Agence française de sécurité sanitaire de l'environnement et du travail (AFSSET), (2008), *Les éthers de glycol : synthèse des connaissances sur les expositions de la population générale et professionnelle en France.*

Agence nationale de sécurité du médicament et des produits de santé (ANSM), (2012), Evaluation du risque lie a l'utilisation du phenoxyethanol dans les produits cosmetiques, 41.

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), (2010), *Exposition de la population générale aux résidus de pesticides en France: Synthèse et recommandations du comité d'orientation et de prospective scientifique de l'observatoire des résidus de pesticides, ORP*.

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), (2019a), *Avis de l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail relatif à une demande d'appui scientifique sur les mesures de protection des riverains lors de l'utilisation des produits phytosanitaires*.

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), (2019b), E-phy database. Retrieved from https://ephy.anses.fr/

Agency for Toxic Substances and Disease Registry (ATSDR), (2019), Toxicological Profiles. Retrieved from https://www.atsdr.cdc.gov/toxprofiledocs/index.html

Aron, A. R., (2007), The Neural Basis of Inhibition in Cognitive Control, *The Neuroscientist*, *133*, 214–228, doi:10.1177/1073858407299288

Ashburner, J., (2007), A fast diffeomorphic image registration algorithm, *NeuroImage*, *381*, 95–113, doi:10.1016/j.neuroimage.2007.07.007

Baar, A. v., Soepatmi, S., Gunning, W. B., & Akkerhuis, G. W., (1994), Development after prenatal exposure to cocaine, heroin and methadone, *Acta Paediatrica*, *83s404*, 40–46, doi:10.1111/j.1651-2227.1994.tb13383.x

Baio, J., (2018), Prevalence of autism spectrum disorder among children aged 8 years — autism and developmental disabilities monitoring network, 11 sites, united states, 2014, *MMWR. Surveillance Summaries*, *67*, doi:10.15585/mmwr.ss6706a1

Bara, A., Manduca, A., Bernabeu, A., Borsoi, M., Serviado, M., Lassalle, O., … Manzoni, O. J., (2018, September 11), Sex-dependent effects of in utero cannabinoid exposure on cortical function, *eLife*, *7*, e36234, doi:10.7554/eLife.36234

Bari, A., & Robbins, T. W., (2013), Inhibition and impulsivity: behavioral and neural basis of response control, *Progress in Neurobiology*, *108Supplement C*, 44–79, doi:10.1016/j.pneurobio.2013.06.005

Barrault, J., (2009), Responsabilité et environnement : questionner l'usage amateur des pesticides, *VertigO*, *Hors série 6*, doi:10.4000/vertigo.8937

Bennett, M. R., Hatton, S., Hermens, D. F., & Lagopoulos, J., (2016), Behavior, neuropsychology and fMRI, *Progress in Neurobiology*, *145-146*, 1–25, doi:10.1016/j.pneurobio.2016.07.001

Béranger, R., Garlantézec, R., Le Maner-Idrissi, G., Lacroix, A., Rouget, F., Trowbridge, J., … Chevrier, C., (2017), Prenatal Exposure to Glycol Ethers and Neurocognitive Abilities in 6-Year-Old Children: The PELAGIE Cohort Study, *Environmental Health Perspectives*, *1254*, 684–690, doi:10.1289/EHP39

Boat, T., & Wu, J., (2015, October 28), *Prevalence of learning disabilities*, National Academies Press (US).

Boyle, C. A., Boulet, S., Schieve, L. A., Cohen, R. A., Blumberg, S. J., Yeargin-Allsopp, M., … Kogan, M. D., (2011), Trends in the Prevalence of Developmental Disabilities in US Children, 1997-2008, *PEDIATRICS*, *1276*, 1034–1042, doi:10.1542/peds.2010-2989

Brietzke, E., Kauer Sant'anna, M., Jackowski, A., Grassi-Oliveira, R., Bucker, J., Zugman, A., … Bressan, R. A., (2012, December), Impact of childhood stress on psychopathology, *Revista Brasileira De Psiquiatria (Sao Paulo, Brazil: 1999)*, *344*, 480–488.

Buck Louis, G. M., Smarr, M. M., & Patel, C. J., (2017, March), The exposome research paradigm: an opportunity to understand the environmental basis for human health and disease, *Current environmental health reports*, *41*, 89–98, doi:10.1007/s40572-017-0126-3

Bundy, J. G., Davey, M. P., & Viant, M. R., (2008, December 28), Environmental metabolomics: a critical review and future perspectives, *Metabolomics*, *51*, 3, doi:10.1007/s11306-008-0152-0

Carrier, G., Brunet, R. C., Bouchard, M., Gosselin, N. H., Dumoulin, M.-J., & Bonvalot, Y., (2005), Évaluation de l'exposition humaine aux organophosphorés et des risques associés à l'aide de biomarqueurs urinaires, 84.

Cartier, C., Warembourg, C., Le Maner-Idrissi, G., Lacroix, A., Rouget, F., Monfort, C., … Chevrier, C., (2016), Organophosphate Insecticide Metabolites in Prenatal and Childhood Urine Samples and Intelligence Scores at 6 Years of Age: Results from the Mother–Child PELAGIE Cohort (France), *Environmental Health Perspectives*, *1245*, 674–680, doi:10.1289/ehp.1409472

Cartier, C., Warembourg, C., Monfort, C., Rouget, F., Limon, G., Durand, G., … Chevrier, C., (2018), Children's contrast sensitivity function in relation to organophosphate insecticide prenatal exposure in the mother-child PELAGIE cohort, *NeuroToxicology*, *67*, 161–168, doi:10.1016/j.neuro.2018.05.004

Chambers, C. D., Garavan, H., & Bellgrove, M. A., (2009), Insights into the neural basis of response inhibition from cognitive and clinical neuroscience, *Neuro-*

science & Biobehavioral Reviews, Translational Aspects of Stopping and Response Control, *335*, 631–646, doi:10.1016/j.neubiorev.2008.08.016

Chen, X. P., Chao, Y. S., Chen, W. Z., & Dong, J. Y., (2017), Mother gestational exposure to organophosphorus pesticide induces neuron and glia loss in daughter adult brain, *Journal of Environmental Science and Health, Part B*, *522*, 77–83, doi:10.1080/03601234.2016.1239973

Cleaton, M., & Kirby, A., (2018), Why Do We Find it so Hard to Calculate the Burden of Neurodevelopmental Disorders, *Journal of Childhood & Developmental Disorders*, *0403*, doi:10.4172/2472-1786.100073

Coker, E., Gunier, R., Bradman, A., Harley, K., Kogut, K., Molitor, J., & Eskenazi, B., (2017), Association between Pesticide Profiles Used on Agricultural Fields near Maternal Residences during Pregnancy and IQ at Age 7 Years, *International Journal of Environmental Research and Public Health*, *145*, 506, doi:10.3390/ijerph14050506

Collignon, O., Girard, S., Gosselin, F., Saint-Amour, D., Lepore, F., & Lassonde, M., (2010), Women process multisensory emotion expressions more efficiently than men, *Neuropsychologia*, *481*, 220–225, doi:10.1016/j.neuropsychologia.2009.09.007

Costa, L. G., (2006), Current issues in organophosphate toxicology, *Clinica Chimica Acta*, *3661*, 1–13, doi:10.1016/j.cca.2005.10.008

Costa, L. G., (2018), Organophosphorus Compounds at 80: Some Old and New Issues, *Toxicological Sciences*, *1621*, 24–35, doi:10.1093/toxsci/kfx266

Costet, N., Béranger, R., Garlantézec, R., Rouget, F., Monfort, C., Cordier, S., ... Chevrier, C., (2018), Occupational exposure to organic solvents during pregnancy and childhood behavior: findings from the PELAGIE birth cohort (France, 2002–2013), *Environmental Health*, *171*, doi:10.1186/s12940-018-0406-x

Coumoul, X., (2017), *Toxicologie*, Sciences Sup, Dunod.

Criaud, M., & Boulinguez, P., (2013), Have we been asking the right questions when assessing response inhibition in go/no-go tasks with fMRI? A meta-analysis and critical review, *Neuroscience & Biobehavioral Reviews*, *371*, 11–23, doi:10.1016/j.neubiorev.2012.11.003

Daneman, R., & Prat, A., (2015), The Blood–Brain Barrier, *Cold Spring Harbor Perspectives in Biology*, *71*, doi:10.1101/cshperspect.a020412

Diagnostic and statistical manual of mental disorders (5th ed.) (American Psychiatric Association), (2013), Washington, DC.

Diamond, A., (2013), Executive Functions, *Annual Review of Psychology*, *641*, 135–168, doi:10.1146/annurev-psych-113011-143750

Diamond, A., (2014), Want to Optimize Executive Functions and Academic Outcomes?, *Minnesota Symposia on Child Psychology*, *37*, 205–232.

Diamond, A., Barnett, W. S., Thomas, J., & Munro, S., (2007), Preschool Program Improves Cognitive Control, *Science (New York, N.Y.) 3185855*, 1387–1388, doi:10.1126/science.1151148

Donauer, S., Altaye, M., Xu, Y., Sucharew, H., Succop, P., Calafat, A. M., ... Yolton, K., (2016), An Observational Study to Evaluate Associations Between Low-Level Gestational Exposure to Organophosphate Pesticides and Cognition During Early Childhood, *American Journal of Epidemiology*, *1845*, 410–418, doi:10.1093/aje/kwv447

Dosenbach, N. U. F., Fair, D. A., Miezin, F. M., Cohen, A. L., Wenger, K. K., Dosenbach, R. A. T., ... Petersen, S. E., (2007), Distinct brain networks for adaptive and stable task control in humans, *Proceedings of the National Academy of Sciences*, *10426*, 11073–11078, doi:10.1073/pnas.0704320104

Drews, U., (1994), *Atlas de poche d'embryologie*, OCLC: 31951741, Paris: Flammarion Médecine-Sciences.

Droutman, V., Bechara, A., & Read, S. J., (2015, November 25), Roles of the different sub-regions of the insular cortex in various phases of the decision-making process, *Frontiers in Behavioral Neuroscience*, *9*, doi:10.3389/fnbeh.2015.00309

Dulcan, M., (1997, October 1), Practice parameters for the assessment and treatment of children, adolescents, and adults with attention-deficit/hyperactivity disorder, *Journal of the American Academy of Child & Adolescent Psychiatry*, *3610*, 85S–121S, doi:10.1097/00004583-199710001-00007

Durston, S., Tottenham, N. T., Thomas, K. M., Davidson, M. C., Eigsti, I.-M., Yang, Y., ... Casey, B., (2003), Differential patterns of striatal activation in young children with and without ADHD, *Biological Psychiatry*, *5310*, 871–878, doi:10.1016/S0006-3223(02)01904-2

Ejaredar, M., Nyanza, E. C., Ten Eycke, K., & Dewey, D., (2015, October), Phthalate exposure and childrens neurodevelopment: a systematic review, *Environmental Research*, *142*, 51–60, doi:10.1016/j.envres.2015.06.014

Encyclopaedia Britannica Publishers, I. S., & Getty Images, I., (2008), *The Encyclopedia Britannica*, OCLC: 276660518.

Eskenazi, B., Gaylord, L., Bracken, M. B., & Brown, D., (1988), In Utero Exposure to Organic Solvents and Human Neurodevelopment, *Developmental Medicine & Child Neurology*, *304*, 492–501, doi:10.1111/j.1469-8749.1988.tb04776.x

Eskenazi, B., Rosas, L. G., Marks, A. R., Bradman, A., Harley, K., Holland, N., ... Barr, D. B., (2008), Pesticide Toxicity and the Developing Brain, *Basic & Clinical Pharmacology & Toxicology*, *1022*, 228–236, doi:10.1111/j.1742-7843.2007.00171.x

European Commission, (2019a), EU Pesticides database. Retrieved from https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN

European Commission, (2019b), Pesticide sales. Retrieved from https://ec.europa.eu/eurostat/web/products-eurostat-news/-/DDN-20181015-1

Forns, J., Torrent, M., Garcia-Esteban, R., Cáceres, A., Pilar Gomila, M., Martinez, D., ... Sunyer, J., (2012), Longitudinal association between early life socio-environmental factors and attention function at the age 11 years, *Environmental Research*, *117*, 54–59, doi:10.1016/j.envres.2012.04.007

Friston, K. J. (Ed.). (2007), *Statistical parametric mapping: the analysis of funtional brain images* (1st ed), Amsterdam ; Boston: Elsevier/Academic Press.

Friston, K. J., Fletcher, P., Josephs, O., Holmes, A., Rugg, M. D., & Turner, R., (1998), Event-Related fMRI: Characterizing Differential Responses, *NeuroImage*, *71*, 30–40, doi:10.1006/nimg.1997.0306

Fromme, H., Nitschke, L., Boehmer, S., Kiranoglu, M., & Göen, T., (2013), Exposure of German residents to ethylene and propylene glycol ethers in general and after cleaning scenarios, *Chemosphere*, *9011*, 2714–2721, doi:10.1016/j.chemosphere.2012.11.051

Furlong, M. A., Engel, S. M., Barr, D. B., & Wolff, M. S., (2014), Prenatal exposure to organophosphate pesticides and reciprocal social behavior in childhood, *Environment International*, *70*, 125–131, doi:10.1016/j.envint.2014.05.011

Furlong, M. A., Herring, A., Buckley, J. P., Goldman, B. D., Daniels, J. L., Engel, L. S., ... Engel, S. M., (2017), Prenatal exposure to organophosphorus pesticides and childhood neurodevelopmental phenotypes, *Environmental Research*, *158*, 737–747, doi:10.1016/j.envres.2017.07.023

Garlantézec, R., Multigner, L., Labat, L., Bonvallot, N., Pulkkinen, J., Dananché, B., ... Cordier, S., (2012), Urinary biomarkers of exposure to glycol ethers and chlorinated solvents during pregnancy: determinants of exposure and comparison with indirect methods of exposure assessment, *Occup Environ Med*, *691*, 62–70, doi:10.1136/oem.2010.062315

Garlantézec, R., Warembourg, C., Monfort, C., Labat, L., Pulkkinen, J., Bonvallot, N., ... Cordier, S., (2013), Urinary Glycol Ether Metabolites in Women and Time to Pregnancy: The PELAGIE Cohort, *Environmental Health Perspectives*, *12110*, 1167–1173, doi:10.1289/ehp.1206103

Germanò, E., Gagliano, A., & Curatolo, P., (2010, September 1), Comorbidity of ADHD and dyslexia, *Developmental neuropsychology*, *35*, 475–93, doi:10.1080/87565641.2010.494748

González-Alzaga, B., Lacasaña, M., Aguilar-Garduño, C., Rodríguez-Barranco, M., Ballester, F., Rebagliato, M., & Hernández, A. F., (2014), A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure, *Toxicology Letters*, Environmental contaminants and target organ toxicities, *2302*, 104–121, doi:10.1016/j.toxlet.2013.11.019

Goyal, M. S., Iannotti, L. L., & Raichle, M. E., (2018), Brain nutrition: a life span approach, *Annual Review of Nutrition*, *381*, 381–399, doi:10.1146/annurev-nutr-082117-051652

Grandjean, P., & Landrigan, P., (2006), Developmental neurotoxicity of industrial chemicals, *The Lancet*, *3689553*, 2167–2178, doi:10.1016/S0140-6736(06)69665-7

Grandjean, P., & Landrigan, P., (2014), Neurobehavioural effects of developmental toxicity, *The Lancet. Neurology*, *133*, 330–338, doi:10.1016/S1474-4422(13)70278-3

Gunier, R. B., Bradman, A., Harley, K. G., Kogut, K., & Eskenazi, B., (2017), Prenatal Residential Proximity to Agricultural Pesticide Use and IQ in 7-Year-Old Children, *Environmental Health Perspectives*, *1255*, 057002, doi:10.1289/EHP504

Gurden, H., (2013), Astrocytes: can they be the missing stars linking neuronal activity to neurofunctional imaging signals?, *Frontiers in Cellular Neuroscience*, *7*, doi:10.3389/fncel.2013.00021

Guxens, M., Lubczyńska, M. J., Muetzel, R. L., Dalmau-Bueno, A., Jaddoe, V. W. V., Hoek, G., … Marroun, H. E., (2018), Air Pollution Exposure During Fetal Life, Brain Morphology, and Cognitive Function in School-Age Children, *Biological Psychiatry*, *00*, doi:10.1016/j.biopsych.2018.01.016

Hankinson, S., Willett, W., Manson, J., Hunter, D., Colditz, G., Stampfer, M., … Speizer, F., (1995), Alcohol, Height, and Adiposity in Relation to Estrogen and Prolactin Levels in Postmenopausal Women, *JNCI Journal of the National Cancer Institute*, *8717*, 1297–1302, doi:10.1093/jnci/87.17.1297

Hasher, L., & Zacks, R. T., (1988), Working Memory, Comprehension, and Aging: A Review and a New View. *in Psychology of Learning and Motivation* (Vol. 22, pp. 193–225), doi:10.1016/S0079-7421(08)60041-9

Horton, M., Kahn, L., Perera, F., Barr, D., & Rauh, V., (2012), Does the home environment and the sex of the child modify the adverse effects of prenatal exposure to chlorpyrifos on child working memory?, *Neurotoxicology and Teratology*, *345*, 534–541, doi:10.1016/j.ntt.2012.07.004

Institut national de l'environnement industriel et des risques (INERIS), (2015), *Ethers de glycol*.

Institut national de la santé et de la recherche (INSERM), (2001), *Alcool : Effets sur la santé*, Les éditions Inserm.

Institut national de la santé et de la recherche (INSERM), (2014), *Pesticides - Effets Sur La Santé.*, OCLC: 875398182, Inserm.

Institut national de la santé et de la recherche médicale (France) (INSERM), (2006), *Éthers de glycol: nouvelles données toxicologiques*, OCLC: 191875357, Paris: INSERM.

Institut national de recherche et de sécurité (INRS), (2011), *Les éthers de glycol*.

Johnson, S., (2007), Cognitive and behavioural outcomes following very preterm birth, *Seminars in Fetal and Neonatal Medicine*, Perinatal Outcomes, *125*, 363–373, doi:10.1016/j.siny.2007.05.004

Julvez, J., & Grandjean, P., (2009), Neurodevelopmental toxicity risks due to occupational exposure to industrial chemicals during pregnancy, *Industrial Health*, *475*, 459–468, doi:10.2486/indhealth.47.459

Jusko, T., van den Dries, M., Pronk, A., Shaw, P., Guxens, M., Spaan, S., ... Longnecker, M., (2019), Organophosphate Pesticide Metabolite Concentrations in Urine during Pregnancy and Offspring Nonverbal IQ at Age 6 Years, *Environmental Health Perspectives*, *1271*, 017007, doi:10.1289/EHP3024

Kastler, B., Vetter, D., Patay, Z., & Germain, P., (2011), *Comprendre l'IRM: manuel d'auto-apprentissage*, OCLC: 779693858, Issy-les-Moulineaux: Elsevier Masson.

Kongtip, P., Techasaensiri, B., Nankongnab, N., Adams, J., Phamonphon, A., Surach, A., ... Woskie, S., (2017), The Impact of Prenatal Organophosphate Pesticide Exposures on Thai Infant Neurodevelopment, *International Journal of Environmental Research and Public Health*, *146*, 570, doi:10.3390/ijerph14060570

Konrad, K., Neufang, S., Hanisch, C., Fink, G. R., & Herpertz-Dahlmann, B., (2006), Dysfunctional attentional networks in children with attention-deficit/hyperactivity–disorder: evidence from an event-related functional magnetic resonance imaging study, *Biological Psychiatry*, *597*, 643–651, doi:10.1016/j.biopsych.2005.08.013

Koureas, M., Tsakalof, A., Tsatsakis, A., & Hadjichristodoulou, C., (2012), Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes, *Toxicology Letters*, Advances on biomonitoring and exposure assessment for pesticides and persistent organic pollutants, *2102*, 155–168, doi:10.1016/j.toxlet.2011.10.007

Kysow, K., Park, J., & Johnston, C., (2017, June), The use of compensatory strategies in adults with ADHD symptoms, *ADHD Attention Deficit and Hyperactivity Disorders*, *92*, 73–88, doi:10.1007/s12402-016-0205-6

Labat, L., Humbert, L., Dehon, B., Multigner, L., Garlantezec, R., Nisse, C., & Lhermitte, M., (1996), Dosage des métabolites urinaires des éthers de glycol par chromatographie en phase gazeuse couplée à la spectrométrie de masse, *Annales de Toxicologie Analytique*, *204*, 227–232, doi:10.1051/ata/2009036

Laslo-Baker, D., Barrera, M., Knittel-Keren, D., Kozer, E., Wolpin, J., Khattak, S., ... Koren, G., (2004), Child Neurodevelopmental Outcome and Maternal Occupational Exposure to Solvents, *Archives of Pediatrics & Adolescent Medicine*, *15810*, 956–961, doi:10.1001/archpedi.158.10.956

Lecendreux, M., Konofal, E., & Faraone, S. V., (2011), Prevalence of Attention Deficit Hyperactivity Disorder and Associated Features Among Children in France, *Journal of Attention Disorders*, *156*, 516–524, doi:10.1177/1087054710372491

Leonard, M., Memmi, S., Rosankis, E., Coutrot, T., Morand, S., Tassy, V., & Sandret, N., (2018), Grossesse et travail : apports de l'enquête SUMER sur les expositions des salariées, *Archives des Maladies Professionnelles et de l'Environnement*, *793*, 305, doi:10.1016/j.admp.2018.03.203

Liddle, P. F., Kiehl, K. A., & Smith, A. M., (2001), Event-related fMRI study of response inhibition, *Human Brain Mapping*, *122*, 100–109, doi:10.1002/1097-0193(200102)12:2<100::AID-HBM1007>3.0.CO;2-6

Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D., & Darnell, J., (2000), Neurotransmitters, Synapses, and Impulse Transmission, *Molecular Cell Biology. 4th edition*.

Lu, C., Toepel ,Kathryn, Irish Rene, Fenske Richard A., Barr Dana B., & Bravo Roberto, (2006), Organic diets significantly lower children's dietary exposure to organophosphorus pesticides, *Environmental Health Perspectives*, *1142*, 260–263, doi:10.1289/ehp.8418

Luna, B., Padmanabhan, A., & O'Hearn, K., (2010), What has fMRI told us about the development of cognitive control through adolescence?, *Brain and Cognition*, *721*, 101–113, doi:10.1016/j.bandc.2009.08.005

Manrique-Garcia, E., Zammit, S., Dalman, C., Hemmingsson, T., Andreasson, S., & Allebeck, P., (2012), Cannabis, schizophrenia and other non-affective psychoses: 35 years of follow-up of a population-based cohort, *Psychological Medicine*, *426*, 1321–1328, doi:10.1017/S0033291711002078

Millenson, M. E., Braun, J. M., Calafat, A. M., Barr, D. B., Huang, Y.-T., Chen, A., ... Yolton, K., (2017), Urinary organophosphate insecticide metabolite concentrations during pregnancy and children's interpersonal, communication, repetitive, and stereotypic behaviors at 8 years of age: The home study, *Environmental Research*, *157*, 9–16, doi:10.1016/j.envres.2017.05.008

Moffitt, T. E., Arseneault, L., Belsky, D., Dickson, N., Hancox, R. J., Harrington, H., ... Caspi, A., (2011), A gradient of childhood self-control predicts health, wealth, and public safety, *Proceedings of the National Academy of Sciences*, *1087*, 2693–2698, doi:10.1073/pnas.1010076108

Möller, T. B., & Reif, E., (2004), *Atlas de poche d'anatomie radiologique*, OCLC: 60174522, Paris: Maloine.

Mortamais, M., Chevrier, C., Philippat, C., Petit, C., Calafat, A. M., Ye, X., ... Slama, R., (2012), Correcting for the influence of sampling conditions on biomarkers of exposure to phenols and phthalates: a 2-step standardization method based on regression residuals, *Environmental Health*, *11*, 29, doi:10.1186/1476-069X-11-29

Mostofsky, S. H., Schafer, J. G. B., Abrams, M. T., Goldberg, M. C., Flower, A. A., Boyce, A., ... Pekar, J. J., (2003), fMRI evidence that the neural basis of response inhibition is task-dependent, *Cognitive Brain Research*, *172*, 419–430, doi:10.1016/S0926-6410(03)00144-7

Moussakova, L., Lachaîne, R., Marieb, E. N., & Hoehn, K., (2014, December 17), *Anatomie et physiologie humaines*, Montréal (Québec): PEARSON.

Muñoz-Quezada, M. T., Lucero, B. A., Barr, D. B., Steenland, K., Levy, K., Ryan, P. B., ... Vega, C., (2013), Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: A systematic review, *NeuroToxicology*, *39*, 158–168, doi:10.1016/j.neuro.2013.09.003

Murray, E., Fernandes, M., Fazel, M., Kennedy, S. H., Villar, J., & Stein, A., (2015), Differential effect of intrauterine growth restriction on childhood neurodevelopment: a systematic review, *BJOG: An International Journal of Obstetrics & Gynaecology*, *1228*, 1062–1072, doi:10.1111/1471-0528.13435

Mustieles, V., Pérez-Lobato, R., Olea, N., & Fernández, M. F., (2015, July), Bisphenol a: human exposure and neurobehavior, *Neurotoxicology*, *49*, 174–184, doi:10.1016/j.neuro.2015.06.002

National Research Council, Division on Earth and Life Studies, Commission on Life Sciences, Board on Environmental Studies and Toxicology, & Committee on Developmental Toxicology, (2000), *Scientific Frontiers in Developmental Toxicology and Risk Assessment*, Google-Books-ID: uTecAgAAQBAJ, National Academies Press.

Nelson, B. K., & Brightwell, W. S., (1984), Behavioral teratology of ethylene glycol monomethyl and monoethyl ethers., *Environmental Health Perspectives*, *57*, 43–46.

Nelson, B. K., Brightwell, W. S., Setzer, J. V., & O'Donohue, T. L., (1984), Reproductive toxicity of the industrial solvent 2-ethoxyethanol in rats and interactive effects of ethanol., *Environmental Health Perspectives*, *57*, 255–259.

Nichols, T., & Hayasaka, S., (2003), Controlling the familywise error rate in functional neuroimaging: a comparative review, *Statistical Methods in Medical Research*, *125*, 419–446, doi:10.1191/0962280203sm341ra

Nougadère, A., Sirot, V., Kadar, A., Fastier, A., Truchot, E., Vergnet, C., ... Leblanc, J.-C., (2012), Total diet study on pesticide residues in France: Levels in food as consumed and chronic dietary risk to consumers, *Environment International*, *45*, 135–150, doi:10.1016/j.envint.2012.02.001

Pelé, F., (2014), *Environnement et développement de l'enfant à 2 ans* (Doctoral dissertation), retrieved from https://ged.univ-rennes1.fr/nuxeo/site/esupversions/95125343-1476-4fca-a16c-2ea3ffb2237d?inline

Pelé, F., Muckle, G., Costet, N., Garlantézec, R., Monfort, C., Multigner, L., ... Cordier, S., (2013), Occupational solvent exposure during pregnancy and child behaviour at age 2, *Occup Environ Med*, *702*, 114–119.

Polańska, K., Jurewicz, J., & Hanke, W., (2015), Smoking and alcohol drinking during pregnancy as the risk factors for poor child neurodevelopment – A review of epidemiological studies, *International Journal of Occupational Medicine and Environmental Health*, *283*, 419–443, doi:10.13075/ijomeh.1896.00424

Power, J. D., Barnes, K. A., Snyder, A. Z., Schlaggar, B. L., & Petersen, S. E., (2012), Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion, *NeuroImage*, *593*, 2142–2154, doi:10.1016/j.neuroimage.2011.10.018

Raffy, G., Mercier, F., Blanchard, O., Derbez, M., Dassonville, C., Bonvallot, N., ... Bot, B. L., (2017), Semi-volatile organic compounds in the air and dust of 30 French schools: a pilot study, *Indoor Air*, *271*, 114–127, doi:10.1111/ina.12288

Rauh, V. A., Garcia, W. E., Whyatt, R. M., Horton, M. K., Barr, D. B., & Louis, E. D., (2015), Prenatal exposure to the organophosphate pesticide chlorpyrifos and childhood tremor, *NeuroToxicology*, *51*, 80–86, doi:10.1016/j.neuro.2015.09.004

Rauh, V. A., Perera, F. P., Horton, M. K., Whyatt, R. M., Bansal, R., Hao, X., ... Peterson, B. S., (2012), Brain anomalies in children exposed prenatally to a common organophosphate pesticide, *Proceedings of the National Academy of Sciences*, *10920*, 7871–7876, doi:10.1073/pnas.1203396109

Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D., & Whyatt, R., (2011), Seven-Year Neurodevelopmental Scores and Prenatal Exposure to Chlorpyrifos, a Common Agricultural Pesticide, *Environmental Health Perspectives*, *1198*, 1196–1201, doi:10.1289/ehp.1003160

Réveillon, M., Urben, S., Barisnikov, K., Borradori Tolsa, C., Hüppi, P. S., & Lazeyras, F., (2013), Functional neuroimaging study of performances on a Go/No-go task in 6- to 7-year-old preterm children: Impact of intrauterine growth restriction, *NeuroImage: Clinical*, *3*, 429–437, doi:10.1016/j.nicl.2013.10.007

Rice, D., & Barone, S., (2000), Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models., *Environmental Health Perspectives*, *108Suppl 3*, 511–533, doi:10.1289/ehp.00108s3511

Rohkamm, R., & Grosshans, B., (2016), *Atlas de poche de neurologie*, OCLC: 948116983, Lavoisier-Médecine sciences.

Rowe, C., Gunier, R., Bradman, A., Harley, K. G., Kogut, K., Parra, K., & Eskenazi, B., (2016), Residential proximity to organophosphate and carbamate pesticide use during pregnancy, poverty during childhood, and cognitive functioning in 10-year-old children, *Environmental Research*, *150*, 128–137, doi:10.1016/j.envres.2016.05.048

Rubia, K., (2013), Functional brain imaging across development, *European Child & Adolescent Psychiatry*, *2212*, 719–731, doi:10.1007/s00787-012-0291-8

Rubia, K., Lim, L., Ecker, C., Halari, R., Giampietro, V., Simmons, A., ... Smith, A., (2013), Effects of age and gender on neural networks of motor response inhi-

bition: from adolescence to mid-adulthood, *NeuroImage*, *83*, 690–703, doi:10.1016/j.neuroimage.2013.06.078

Rubia, K., Russell, T., Overmeyer, S., Brammer, M. J., Bullmore, E. T., Sharma, T., ... Taylor, E., (2001), Mapping Motor Inhibition: Conjunctive Brain Activations across Different Versions of Go/No-Go and Stop Tasks, *NeuroImage*, *132*, 250–261, doi:10.1006/nimg.2000.0685

Sagiv, S. K., Bruno, J. L., Baker, J. M., Palzes, V., Kogut, K., Rauch, S., ... Eskenazi, B., (2019, August 21), Prenatal exposure to organophosphate pesticides and functional neuroimaging in adolescents living in proximity to pesticide application, *Proceedings of the National Academy of Sciences*, 201903940, doi:10.1073/pnas.1903940116

Sanders, A. P., Claus Henn, B., & Wright, R. O., (2015), Perinatal and Childhood Exposure to Cadmium, Manganese, and Metal Mixtures and Effects on Cognition and Behavior: A Review of Recent Literature, *Current Environmental Health Reports*, *23*, 284–294, doi:10.1007/s40572-015-0058-8

Santé publique France, (2019), Imprégnation de la population française par les éthers de glycol. Programme national de biosurveillance, Esteban 2014-2016, 46.

Schleier, J. J., & Peterson, R. K. D., (2011), CHAPTER 3:Pyrethrins and Pyrethroid Insecticides. *in Green Trends in Insect Control* (pp. 94–131), doi:10.1039/9781849732901-00094

Schug, T. T., Blawas, A. M., Gray, K., Heindel, J. J., & Lawler, C. P., (2015), Elucidating the Links Between Endocrine Disruptors and Neurodevelopment, *Endocrinology*, *1566*, 1941–1951, doi:10.1210/en.2014-1734

Sharma, B. M., Sáňka, O., Kalina, J., & Scheringer, M., (2019), An overview of worldwide and regional time trends in total mercury levels in human blood and breast milk from 1966 to 2015 and their associations with health effects, *Environment International*, *125*, 300–319, doi:10.1016/j.envint.2018.12.016

Sheridan, M. A., & McLaughlin, K. A., (2014, November), Dimensions of early experience and neural development: deprivation and threat, *Trends in Cognitive Sciences*, *1811*, 580–585, doi:10.1016/j.tics.2014.09.001

Silver, M. K., Shao, J., Ji, C., Zhu, B., Xu, L., Li, M., ... Meeker, J. D., (2018), Prenatal organophosphate insecticide exposure and infant sensory function, *International Journal of Hygiene and Environmental Health*, *2213*, 469–478, doi:10.1016/j.ijheh.2018.01.010

Silver, M. K., Shao, J., Zhu, B., Chen, M., Xia, Y., Kaciroti, N., ... Meeker, J. D., (2017), Prenatal naled and chlorpyrifos exposure is associated with deficits in infant motor function in a cohort of Chinese infants, *Environment International*, *106*, 248–256, doi:10.1016/j.envint.2017.05.015

Simmonds, D. J., Pekar, J. J., & Mostofsky, S. H., (2008), Meta-analysis of Go/No-go tasks demonstrating that fMRI activation associated with response inhi-

bition is task-dependent, *Neuropsychologia*, *461*, 224–232, doi:10.1016/j.neuropsychologia.2007.07.015

Simon, G., Lubin, A., Houdé, O., & De Neys, W., (2015, October 2), Anterior cingulate cortex and intuitive bias detection during number conservation, *Cognitive Neuroscience*, *64*, 158–168, doi:10.1080/17588928.2015.1036847

Sinn, N., (2008), Nutritional and dietary influences on attention deficit hyperactivity disorder, *Nutrition Reviews*, *6610*, 558–568, doi:10.1111/j.1753-4887.2008.00107.x

Slotkin, T. A., & Seidler, F. J., (2005), The alterations in CNS serotonergic mechanisms caused by neonatal chlorpyrifos exposure are permanent, *Developmental Brain Research*, *1581*, 115–119, doi:10.1016/j.devbrainres.2005.06.008

Smit, F., Bolier, L., & Cuijpers, P., (2004), Cannabis use and the risk of later schizophrenia: a review, *Addiction*, *994*, 425–430, doi:10.1111/j.1360-0443.2004.00683.x

Spierer, L., Chavan, C. F., & Manuel, A. L., (2013, August 1), Training-induced behavioral and brain plasticity in inhibitory control, *Frontiers in Human Neuroscience*, *7*, doi:10.3389/fnhum.2013.00427

Stewart, P., Fitzgerald, S., Reihman, J., Gump, B., Lonky, E., Darvill, T., ... Hauser, P., (2003), Prenatal PCB exposure, the corpus callosum, and response inhibition., *Environmental Health Perspectives*, *11113*, 1670–1677.

Sudakin, D. L., & Stone, D. L., (2011), Dialkyl phosphates as biomarkers of organophosphates: The current divide between epidemiology and clinical toxicology, *Clinical Toxicology*, *499*, 771–781, doi:10.3109/15563650.2011.624101

Suskauer, S. J., Simmonds, D. J., Fotedar, S., Blankner, J. G., Pekar, J. J., Denckla, M. B., & Mostofsky, S. H., (2008), Functional magnetic resonance imaging evidence for abnormalities in response selection in attention deficit hyperactivity disorder: differences in activation associated with response inhibition but not habitual motor response, *Journal of cognitive neuroscience*, *203*, 478–493, doi:10.1162/jocn.2008.20032

Susser, E., Hoek, H. W., & Brown, A., (1998), Neurodevelopmental Disorders after Prenatal Famine: The Story of the Dutch Famine Study, *American Journal of Epidemiology*, *1473*, 213–216, doi:10.1093/oxfordjournals.aje.a009439

Swedo, S. E., Seidlitz, J., Kovacevic, M., Latimer, M. E., Hommer, R., Lougee, L., & Grant, P., (2015, February 1), Clinical presentation of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections in research and community settings, *Journal of Child and Adolescent Psychopharmacology*, *251*, 26–30, doi:10.1089/cap.2014.0073

Tata, A. M., Velluto, L., D'Angelo, C., & Reale, M., (2014), Cholinergic system dysfunction and neurodegenerative diseases: cause or effect?, *CNS & neurological disorders drug targets*, *137*, 1294–1303.

Terry, A. V., (2012), Functional consequences of repeated organophosphate exposure: Potential non-cholinergic mechanisms, *Pharmacology & Therapeutics*, *1343*, 355–365, doi:10.1016/j.pharmthera.2012.03.001

Thompson, W., Russell, G., Baragwanath, G., Matthews, J., Vaidya, B., & Thompson-Coon, J., (2018, April), Maternal thyroid hormone insufficiency during pregnancy and risk of neurodevelopmental disorders in offspring: a systematic review and meta-analysis, *Clinical Endocrinology*, *884*, 575–584, doi:10.1111/cen.13550

Thurman, D. J., (2016), The Epidemiology of Traumatic Brain Injury in Children and Youths: A Review of Research Since 1990, *Journal of Child Neurology*, *311*, 20–27, doi:10.1177/0883073814544363

Tie, Y., Suarez, R. O., Whalen, S., Radmanesh, A., Norton, I. H., & Golby, A. J., (2009), Comparison of blocked and event-related fMRI designs for pre-surgical language mapping, *NeuroImage*, *47 Suppl 2*, T107–T115, doi:10.1016/j.neuro\-ima\-ge.2008.11.020

Till, C., Koren, G., & Rovet, J., (2001), Prenatal exposure to organic solvents and child neurobehavioral performance, *Neurotoxicology and Teratology*, Twenty-fifth annual meeting of the Neurobehavioral Teratology Society, *233*, 235–245, doi:10.1016/S0892-0362(01)00141-6

Till, C., Westall, C. A., Rovet, J. F., & Koren, G., (2001), Effects of maternal occupational exposure to organic solvents on offspring visual functioning: a prospective controlled study, *Teratology*, *643*, 134–141, doi:10.1002/tera.1056

Till, C., Westall, C., Koren, G., Nulman, I., & Rovet, J., (2005), Vision abnormalities in young children exposed prenatally to organic solvents, *Neurotoxicology*, *264*, 599–613, doi:10.1016/j.neuro.2005.05.011

Tran, N. Q. V., & Miyake, K., (2017), Neurodevelopmental Disorders and Environmental Toxicants: Epigenetics as an Underlying Mechanism, *International Journal of Genomics*, *2017*, doi:10.1155/2017/7526592

Tworoger, S. S., & Hankinson, S. E., (2006), Use of biomarkers in epidemiologic studies: minimizing the influence of measurement error in the study design and analysis, *Cancer Causes & Control*, *177*, 889–899, doi:10.1007/s10552-006-0035-5

Vernet, C., Philippat, C., Calafat, A. M., Ye, X., Lyon-Caen, S., Siroux, V., ... Slama, R., (2018), Within-Day, Between-Day, and Between-Week Variability of Urinary Concentrations of Phenol Biomarkers in Pregnant Women, *Environmental Health Perspectives*, *1263*, doi:10.1289/EHP1994

Vinck, L., & Memmi, S., (2010), *Les expositions aux risques professionnels Les produits chimiques Enquête Sumer 2010*.

Vineis, P., (1997), Sources of variation in biomarkers, *IARC scientific publications*, *142*, 59–71.

Vrijheid, M., Casas, M., Gascon, M., Valvi, D., & Nieuwenhuijsen, M., (2016), Environmental pollutants and child health—A review of recent concerns, *International Journal of Hygiene and Environmental Health*, *2194-5*, 331–342, doi:10.1016/j.ijheh.2016.05.001

Wang, Y., Zhang, Y., Ji, L., Hu, Y., Zhang, J., Wang, C., ... Tian, Y., (2017), Prenatal and postnatal exposure to organophosphate pesticides and childhood neurodevelopment in Shandong, China, *Environment International*, *108*, 119–126, doi:10.1016/j.envint.2017.08.010

Wellcome Centre for Human Neuroimaging, (2019), Statistical Parametric Mapping (SPM). Retrieved from https://www.fil.ion.ucl.ac.uk/spm/

Wessels, D., Barr, D. B., & Mendola, P., (2003), Use of biomarkers to indicate exposure of children to organophosphate pesticides: implications for a longitudinal study of children's environmental health., *Environmental Health Perspectives*, *11116*, 1939–1946.

White, R. F., Palumbo, C. L., Yurgelun-Todd, D. A., Heaton, K. J., Weihe, P., Debes, F., & Grandjean, P., (2011), Functional MRI approach to developmental methylmercury and polychlorinated biphenyl neurotoxicity, *Neurotoxicology*, *326*, 975–980, doi:10.1016/j.neuro.2011.04.001

Wolburg, H., & Lippoldt, A., (2002), Tight junctions of the blood–brain barrier: development, composition and regulation, *Vascular Pharmacology*, Blood Brain Barrier, *386*, 323–337, doi:10.1016/S1537-1891(02)00200-8

Yang, H., Wu, Q.-Z., Guo, L.-T., Li, Q.-Q., Long, X.-Y., Huang, X.-Q., ... Gong, Q.-Y., (2011), Abnormal spontaneous brain activity in medication-naïve ADHD children: A resting state fMRI study, *Neuroscience Letters*, *5022*, 89–93, doi:10.1016/j.neulet.2011.07.028

Ye, B. S., Leung, A. O. W., & Wong, M. H., (2017, August), The association of environmental toxicants and autism spectrum disorders in children, *Environmental Pollution (Barking, Essex: 1987)*, *227*, 234–242, doi:10.1016/j.envpol.2017.04.039

Yolton, K., Cornelius, M., Ornoy, A., McGough, J., Makris, S., & Schantz, S., (2014), Exposure to neurotoxicants and the development of attention deficit hyperactivity disorder and its related behaviors in childhood, *Neurotoxicology and Teratology*, *44*, 30–45, doi:10.1016/j.ntt.2014.05.003

Zhang, Y., Han, S., Liang, D., Shi, X., Wang, F., Liu, W., ... Tian, Y., (2014), Prenatal Exposure to Organophosphate Pesticides and Neurobehavioral Development of Neonates: A Birth Cohort Study in Shenyang, China, *PLOS ONE*, *92*, e88491, doi:10.1371/journal.pone.0088491

Figures

Criaud, M., & Boulinguez, P., (2013), Have we been asking the right questions when assessing response inhibition in go/no-go tasks with fMRI? A meta-analysis and critical review, *Neuroscience & Biobehavioral Reviews*, *371*, 11–23, doi:10.1016/j.neubiorev.2012.11.003

Encyclopaedia Britannica Publishers, I. S., & Getty Images, I., (2008), *The Encyclopedia Britannica*, OCLC: 276660518.

Kastler, B., Vetter, D., Patay, Z., & Germain, P., (2011), *Comprendre l'IRM: manuel d'auto-apprentissage*, OCLC: 779693858, Issy-les-Moulineaux: Elsevier Masson.

Moussakova, L., Lachaîne, R., Marieb, E. N., & Hoehn, K., (2014, December 17), *Anatomie et physiologie humaines*, Montréal (Québec): PEARSON.

Pradal, G., (2005), *Embryologie humaine élémentaire*, Ellipses.

Rice, D., & Barone, S., (2000), Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models., *Environmental Health Perspectives*, *108Suppl 3*, 511–533, doi:10.1289/ehp.00108s3511

Wellcome Centre for Human Neuroimaging, (2019), Statistical Parametric Mapping (SPM). Retrieved from https://www.fil.ion.ucl.ac.uk/spm/

All atomic nuclei consist of protons and neutrons, with a net positive charge. Certain atomic nuclei, such as the hydrogen nucleus (1H), possess a property known as “spin”, dependent on the odd number of protons and/or neutrons (for hydrogen, one unique proton and no neutron). This can be conceived as the nucleus spinning around its own axis, generating a local magnetic field with north and south poles. Main nuclei of biological interest are hydrogen nucleus (1H), carbon isotope (13C), fluorine nucleus (19F), phosphorus nucleus (31P) and sodium nucleus (23 Na). Due to its natural abundance and its high intrinsic magnetic moment, hydrogen nucleus, or proton, plays the most important role in MRI, also called proton MRI. Nuclei possessing a spin are candidates to NMR. In presence of a strong external magnetic field (hereafter referred as B0), nuclear spins will be aligned with B0 with two energy states: a low-energy state (parallel to the magnetic field) or a high-energy state (anti-parallel to the magnetic field direction). There is a small excess of spins in the same direction of B0, sufficient to produce a NMR signal at tissue level. Besides, the nucleus spinning around its own axis generates a local magnetic field with north and south poles. The precessing velocity, also called Larmor frequency, is described by the Larmor equation, $\omega_o = \gamma B0$: it is proportional to the field strength[21].
Within the static magnetic field, nuclei can be excited by the application of a second magnetic field (hereafter referred as B1) perpendicular to B0 during short pulses of a few microseconds. Nuclei absorb the energy and transit from lower to higher energy states. The system then returns to equilibrium in a process called relaxation. This return to equilibrium can be detected and is called the free-induction decay (FID).
Relaxation is the term used to describe the process by which a nuclear spin returns to thermal equilibrium after absorbing energy. There are two types of relaxation, longitudinal and transverse relaxations, and these are described by the time constants, T1 and T2, respectively.
Each tissue returns to its equilibrium state after excitation by the independent relaxation processes of T1 and T2. Contrast can be manipulated by varying the times between RF pulses, the repetition time (TR) and the echo time (TE). Thus, water and CSF that have long T1 values (3000–5000 ms), appear dark on T1-weighted images, while fat, present in WM has a short T1 value (260 ms) and appears bright. In an idealized system, all nuclei relax with the same frequency. However, in real systems, there are minor differences in chemical environment that can disperse the distribution of resonance frequencies around the ideal. This results in T2*-weighted imaging.

21. Kastler et al., *Comprendre l'IRM*

ADHD diagnostic is based before age 7 years if six of the following items are positive during more than 6 months:

- Shortness of attention:
  - No attention for details or careless mistakes
  - Short span of attention during playing or tasks
  - Seems not to listen when he/she is spoken to
  - Doesn't do what he/she is said or is not finishing tasks
  - Difficulties in organizing tasks or activities
  - Avoids or hates tasks that need longer attention
  - Loses often things
  - Easily distracted by external stimulation
  - Is very forgetful in daily tasks
- Hyperactivity
  - Moves often with hands and foots
  - Cannot sit still when he/she is expected to
  - Is running around and climbs on everything
  - Difficulties in quiet playing or relaxing activities
  - Is often busy and goes on and on
  - Talks without stopping
- Impulsiveness
  - Answers before the question is ended
  - Difficulties in waiting in his/her turn
  - Often disturbs others or is intrusive

Molecule	Authorization in France (in 2019)	Year of withdrawal	DAP Metabolites
Acephate	No	2003	DMP, DMTP
Azamethiphos	No	2002	DMP, DMTP
Azinphos ethyl	No	1995	DEP, DETP, DEDTP
Azinphos-methyl	No	2005	DMP, DMTP, DMDTP
Bromophos	No	2002	DMP, DMTP
Bromophos-ethyl	No	2002	DEP, DETP
Cadusafos	No	2007	DEP, DETP, DEDTP
Carbophenothion	No	2002	DEP, DETP, DEDTP
Chlorfenvinphos	No	2002	DEP
Chlormephos	No	2002	DEP, DETP, DEDTP
Chlorpyrifos	Yes		DEP, DETP
Chlorpyrifos-methyl	Yes		DMP, DMTP
Dialifos	No	2002	DEP, DETP, DEDTP
Diazinon	No	2007	DEP, DETP
Dichlofenthion	No	2002	DEP, DETP
Dichlorvos	No	2007	DMP
Dimefox	No	2002	DMP
Dimethoate	No	2019	DMP, DMTP, DMDTP
Dioxathion	No	2002	DEP, DETP, DEDTP
Disulfoton	No	2002	DEP, DETP, DEDTP
Ditalimfos	No	2002	DEP, DETP
Ethoprophos	No	2019	DEP, DETP, DEDTP
Etrimfos	No	2002	DMP, DMTP
Fenamiphos	Yes		DEP
Fenitrothion	No	2007	DMP, DMTP
Fenthion	No	2004	DMP, DMTP
Fonofos	No	2002	DEP, DETP, DEDTP
Formothion	No	2002	DMP, DMTP, DMDTP
Fosthiazate	Yes		DEP, DETP
Heptenophos	No	2002	DMP
Iodofenphos	No	2002	DMP, DMTP
Isazofos	No	2002	DMP, DMTP
Isofenphos	No	2002	DEP, DETP, DEDTP

Continued on next page

Molecule	Authorization in France (in 2019)	Year of withdrawal	DAP Metabolites
Malathion	Yes		DMP, DMTP, DMDTP
Mecarbam	No	2002	DEP, DETP, DEDTP
Methamidophos	No	2006	DMP, DMTP
Methidathion	No	2004	DMP, DMTP, DMDTP
Mevinphos	No	2002	DMP
Monocrotophos	No	2002	DMP
Naled	No	2005	DMP
Omethoate	No	2002	DMP, DMTP
Oxydemeton-methyl	No	2007	DMP, DMTP
Parathion	No	2001	DEP, DETP
Parathion-methyl	No	2003	DMP, DMTP
Phorate	No	2002	DEP, DETP, DEDTP
Phosalone	No	2006	DEP, DETP
Phosmet	Yes		DMP, DMTP, DMDTP
Phosphamidon	No	2002	DMP
Phoxim	No	2007	DEP, DETP
Pirimiphos-methyl	Yes		DMP, DMTP
Profenofos	No	2002	DEP, DETP
Propetamphos	No	2002	DMP, DMTP
Prothoate	No	2002	DEP, DETP
Pyraclofos	No	2002	DEP, DETP
Pyrazophos	No	2000	DEP, DETP
Quinalphos	No	2002	DEP, DETP
Sulfotep	No	2002	DEP, DETP
Temephos	No	2002	DMP, DMTP
Terbufos	No	2002	DEP, DETP, DEDTP
Tetrachlorvinphos	No	2002	DMP
Thiometon	No	2002	DMP, DMTP, DMDTP
Tolclofos-methyl	Yes		DMP, DMTP
Trichlorfon	No	2007	DMP
Trichloronate	No	2002	DEP, DETP
Vamidothion	No	2002	DMP, DMTP, DMDTP

UNIVERSITE BIOLOGIE BRETAGNE SANTE LOIRE

UNIVERSITÉ DE RENNES 1

Effets de l'exposition prénatale aux neurotoxiques sur le fonctionnement du cerveau de l'enfant évalué par imagerie cérébrale

Mot clés : *Neurodéveloppement, expositions prénatales, biomarqueurs, IRM, inhibition motrice, cohorte*

Résumé : La vulnérabilité du cerveau en développement à son environnement est largement décrite dans la littérature. Certains effets cognitifs et comportementaux observés après une exposition prénatale aux éthers de glycol et aux insecticides organophosphorés suggèrent une possible atteinte des fonctions exécutives. Nous avons notamment suspecté une altération des capacités d'inhibition, une fonction prédictrice des performances scolaires et des comportements à risque à l'âge adulte. Ainsi, améliorer notre compréhension de ces effets semble être un enjeu majeur de santé publique. L'objectif de la thèse était d'évaluer les effets de l'exposition prénatale aux éthers de glycol et aux organophosphorés sur le fonctionnement du cerveau de l'enfant évalué par imagerie cérébrale. Ce travail s'appuie sur les données de la cohorte mère-enfant PELAGIE. Le suivi longitudinal a permis de mesurer l'exposition aux neurotoxiques pendant la grossesse par biomarqueurs. Nous avons utilisé une tâche de Go/No-Go et l'IRM fonctionnelle pour évaluer le contrôle inhibiteur et ses mécanismes neuraux chez des enfants âgés de 10 à 12 ans. Nos résultats suggèrent que le contrôle inhibiteur pourrait être altéré après une exposition prénatale à ces contaminants. Cependant, certains résultats concernant les éthers de glycol sont inattendus au regard de la littérature et peu robustes, ce qui ne nous permet pas de conclure à un effet important sur le cerveau en développement. Enfin, nous avons suggéré que le cortex frontal, impliqué dans le réseau de l'inhibition, peut être une cible de l'effet des organophosphorés.

Effects of prenatal exposure to neurotoxicants on the child's brain function evaluated by cerebral imaging

Keywords : *Neurodevelopment, prenatal exposures, biomarkers, MRI, motor inhibition, cohort*

Abstract: The vulnerability of the developing brain to its environment is well known in the literature. Some cognitive and behavioral alterations observed after prenatal exposure to glycol ethers and organophosphate insecticide suggest a possible impairment of executive functions. In particular, we suspected an alteration of the inhibitory control, a predictor of academic performance or tendency to risky behaviors in adulthood. Therefore, better understanding these effects appears as a key issue of public health. We aimed at investigating the effects of prenatal exposure to glycol ethers and organophosphate insecticides on the child's brain function evaluated by cerebral imaging. This work is based on the data of the mother-child PELAGIE cohort. The longitudinal follow-up allowed to measure with biomarkers the exposure to neurotoxicants during pregnancy. We used a Go/No-Go task and functional MRI to assess the inhibitory control and its neural mechanisms in children aged 10-12 years. Our results suggest that inhibitory control may be altered after prenatal exposure to these contaminants. However, some of our findings about glycol ethers were unexpected regarding existing literature and showed little consistency, preventing us to conclude to a significant effect on the developing brain. Then, we suggested that the frontal cortex, involved in inhibition network, may be a specific target of organophosphates.