*2. Methods*

*2.1 Participants*

From prior cohort studies, 59 mother–child couples were recruited in the middle of Taiwan and included in the study {Lien, 2015 #81}{Wang, 2015 #82}. After obtaining an in-depth explanation of this research, the questionnaire was completed. It contained questions regarding basic demographics, food patterns throughout pregnancy, and medical history. Pregnant women's urine and/or serum samples were obtained during the third trimester to assess prenatal EDC exposure levels. We collected the brain imaging of the teenage children (male/female: 33/26, mean age: 13.95±0.47 years) of the mothers mentioned above from authorised hospitals. We acquired 59 magnetic resonance imaging (MRI) scans using 3 T MRI scanners at the Chung Shan Medical University Hospital. Teenage serum samples were also obtained during the third trimester to assess prenatal EDC exposure levels. During this period, the women took additional questionnaires that requested information about their education level, occupation, and family income.

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*2.2 EDC measurements*

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*2.3 Imaging acquisition*

All participants underwent a whole-brain volumetric T1-weighted images and diffusion-weighted scan acquired using a 20-channel head coil on the 3T imaging system (Skyra, Siemens, Germany).

The 3D T1-weighted images were obtained using an magnetization-prepared rapid gradient-echo (MPRAGE) sequence with the following parameters: matrix 256 × 256; 160 slices; 1 mm in plane resolution, slice thickness=1 mm; TE/TR = 2.27/2500 ms; TI = 902 ms; flip angle = 8◦.

Diffusion-weighted images were acquired using echo-planar imaging (SE-EPI), with TE/TR=97/4800 ms; 128 × 128 acquisition matrix and 35 slices; voxel size (resolution) =2 × 2 × 4 mm3; 64 isotopically distributed orientations for the diffusion weighted gradient at b-value = 1000, 1500, 2000 s/mm2, and 12 b=0 s/mm2 images.

*2.4 TI Image processing*

T1 images were processed using FreeSurfer (Version 6) automated neuroanatomical segmentation software2. Cortical thickness and surface area values were determined using the recon-all script of FreeSurfer version 5.3 (Fischl, 2012) on each T1-weighted scan [1]. The processing using FreeSurfer includes motion correction, the removal of non-brain tissue, intensity normalization, applied affine transformation to Montreal Neurological Institute(MNI) space, Talairach alignment, who brain segmentation [2],cortical surface reconstruction [3, 4] and the cerebral cortex parcellation [5]. After segmentation, all outputs were checked by visual assessment, removal of non-brain tissue was performed using FSL’s *bet*, and re-runed the recon-all scripts. The cortical thickness is estimated using FreeSurfer by finding the shortest path between each vertex of the white matter surface and the gray matter surface. The shortest distance between this vertex of the gray matter surface and the white matter surface is determined. At last, we estimate the cortical thickness as the average of these two distances [4]. Mean Surface areas and mean cortical thicknesses in each region of interest (ROI) of FreeSurfer's atlas were obtained from FreeSurfer's output *\*.aparc.stats* files.

*2.5 DKI Image preprocessing*

First, diffusion weighted image was denoised by using Marchenko-Pastur principal component analysis (MP-PCA) denoising[6]. Gibbs-ringing artifact was eliminated by using the method of local subvoxel-shifts proposed by Kellner et al. [7]. Eddy current induced distortion and motion artifacts were corrected by FSL’s *eddy* [8, 9]. DKI metrics were then generated for mean kurtosis (MK), axial kurtosis (AK) and radial kurtosis (RK) by using *DIPY* software [10]. We used the JHU DTI-based white matter atlas (ICBM-DTI-81 white matter labels atlas) [11] to perform the parcellation method described by Lo et al. [12]. The JHU white matter template was applied to identify the regions in white matter for determining DKI metrics. The DKI metrics extracted from each white matter tract averaged across the all voxels for all subjects.

*2.6 Statistical analysis*

*2.6.1 Demographic statistic*

Statistical analyses were carried out using Statistical Product and Service Solutions (IBM SPSS Statistics Version 20.0. Armonk, NY).

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*2.6.2 Statistical analysis of images*

Multivariate linear regression was performed to find the association between EDC concentration and the cortical measurements of teenage brain. To eliminate multiple-comparison effects, Bonferroni correction (0.05/68) was performed for 68 cortical regions. A Bonferroni-corrected *p*-value of less than 0.05 was considered as statistically significant.

The connection between EDC levels and the DKI metrics of teenage white matter tracts was determined using multivariate linear regression. To eliminate multiple-comparison effects, Bonferroni correction (0.05/48) was performed for 48 white matter tracts. A *p*-value less than 0.05 after Bonferroni correction was considered statistically significant.

To reduce the impact from other confounders, family income and gender were selected and applied in the model as covariates. In order to minimize the effect of other confounders, xxx and xxx were chosen and included as covariates in the analysis. Additionally, we divided boys and girls into distinct groups for multivariate regression analyses in which xxx was retained as a covariate. Different concentrations of EDCs were used as covariates to separate the impacts of other EDCs. Significant regions (Bonferroni-corrected *p* < 0.05) were illustrated.

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