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Sensitivity of continuous performance test (CPT) at age 14 years to developmental methylmercury exposure

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ABSTRACT

Hit Reaction Time latencies (HRT) in the Continuous Performance Test (CPT) measure the speed of visual information processing. The latencies may involve different neuropsychological functions depending on the time from test initiation, i.e., first orientation, learning and habituation, then cognitive processing and focused attention, and finally sustained attention as the dominant demand. Prenatal methylmercury exposure is associated with increased reaction time (RT) latencies. We therefore examined the association of methylmercury exposure with the average HRT at age 14 years at three different time intervals after test initiation. A total of 878 adolescents (87% of birth cohort members) completed the CPT. The RT latencies were recorded for 10 min, with visual targets presented at 1000 ms intervals. After confounder adjustment, regression coefficients showed that CPT-RT outcomes differed in their associations with exposure biomarkers of prenatal methylmercury exposure: During the first 2 min, the average HRT was weakly associated with methylmercury (beta (SE) for a ten-fold increase in exposure, (3.41 (2.06)), was strongly for the 3-to-6 min interval (6.10 (2.18)), and the strongest during 7–10 min after test initiation (7.64 (2.39)). This pattern was unchanged when simple reaction time and finger tapping speed were included in the models as covariates. Postnatal methylmercury exposures did not affect the outcomes. Thus, these findings suggest that sustained attention as a neuropsychological domain is particularly vulnerable to developmental methylmercury exposure, indicating probable underlying dysfunction of the frontal lobes. When using CPT data as a possible measure of neurotoxicity, test results should therefore be analyzed in regard to time from test initiation and not as overall average reaction times.

1. Introduction

The Continuous Performance Test Hit Reaction Time (CPT-HRT) is often used to assess attention functioning along with other CPT outcomes such as errors of omission and commission [7,9,12,13,30,31]. Some authors have suggested that omissions are more likely to be related to inattention symptoms and commissions to hyperactivity–impulsivity symptoms [4,12,13]. However, it is less clear if CPT-HRT outcomes, in terms of the latency averages, may be related to attention disorders, e.g., ADHD symptoms [13].

From a neuropsychological perspective, the CPT-HRT is considered a higher-order cognitive function [14] involving the speed in processing visual information [8,25,26]. As the HRT is measured only when the target stimulus has been presented on the screen, the identification involves working memory and a subsequent decision to activate the hand muscles to press the button. Pressing the button at other times suggests problems of inhibition and is expressed as a commission error outcome without a HRT [10,13]. Omission errors may be caused by at least two different neuropsychological functions, i.e., a true distraction from the target, or a slow HRT response after the brain identified the target stimulus, but the information was processed too slowly for the time limit before the next stimulus appeared on the screen [10,13]. In general, neuropsychological theory has not been applied in the analysis of HRT data from CPT studies. The lack of a more elaborate conceptualization suggests the need for further scrutiny of CPT data to improve the interpretation of neuropsychological functions underlying this particular task.

We separated CPT-HRT results into three mental stages during which we argue that different functional domains are dominant. The first few minutes are thought to be primarily influenced by attitudinal factors related to orientation, learning and habituation. Some authors have skipped this stage of data analyses due to anticipated noise in regard to the desired cognitive task, i.e., attention functioning [10,11,16]. A subsequent stage involves speed processing and selective

Abbreviations: NES2, Neurobehavioral Evaluation System; CPT-HRT, Continuous Performance Task Hit Reaction Time; RT, Reaction Time; ADHD, Attention Deficit Hyperactivity Disorder; Hg, Mercury; MS, Milliseconds.

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focused attention as a primary functional domain [27]. At this stage, the subject's performance may not yet be influenced by mental fatigue from this simple and repetitive task. In the final stage, the HRT latencies are more affected by sustained attention as a dominant demand. At this stage, motivation and capability (i.e., cognitive control) to maintain the selective focused attention functioning seem to be the major issue. These stages are not mutually exclusive since the task remains unchanged. Rather, it is the neuropsychological demand that changes over time. Other more basic neuropsychological functions, such as motor speed and simple reaction time, are also involved in all three hypothetical stages, although they are unlikely to change with time [26].

Prenatal methylmercury exposure has been linked to an increase in CPT-HRT latencies in a birth cohort from the Faroe Islands examined at ages 7 and 14 years. Negative associations with other neuropsychological domains, including verbal, visuospatial, and motor functions were also reported [11]. The present study utilizes the same cohort data.

The objective of this study was to assess prenatal and postnatal methylmercury exposures and their association with the three stages of CPT-HRT latencies in 14-year olds. Using the same instrument and task to assess different neuropsychological functions provides standardized outcomes and helps to avoid noise derived from specific psychometric characteristics when administering different tests.

2. Materials and methods

The cohort was assembled in the Faroe Islands during a 21-month period in 1986–1987 [15]. The primary indicators of intrauterine exposure to methylmercury were mercury concentrations assessed in cord blood and in maternal hair at parturition. Methylmercury exposure was found to vary considerably: 15% of the mothers had hair mercury concentrations above 10 μg/g, while 4% were below 1 μg/g, a level that corresponds to the exposure limit recommended by the U.S. Environmental Protection Agency [5,29].

Cohort members were first invited for a detailed examination at school age (7 years), when a total of 917 eligible children (90.3%) participated [16]. Subsequently, a total of 878 of the 1010 living 14-year old cohort members (86.9%) were examined later [11]. Most examinations took place at the National Hospital in Tórshavn, the capital of the Faroe Islands, during April–June of 2000 and 2001. For families who had moved to Denmark, examinations were also offered in Odense in November 2000. Transportation costs and other expenses were reimbursed in accordance with rates approved by the Faroese Ethical Review Committee. A total of eight participants were examined per day, four in the morning and four in the afternoon. Each group of participants rotated between four stations staffed by health professionals who administered the clinical tests with no access to information on individual exposure levels. Approximately one hour was spent at each station for a physical examination, neurophysiologic tests, and a series of two neuropsychological tests, each of which was administered by the same examiner in the same sequence throughout. The thorough pediatric examination included an assessment of neurological function. A total of 18 participants examined had neurological disorders thought to be independent of methylmercury exposure and thus were excluded from the data analysis. The methylmercury exposure of these participants did not differ from that of other cohort members. Of the remaining 860 participants who completed the 14-year examination schedule, 815 had also been examined at age 7. Blood specimens and hair samples were again obtained, and the proximal 2-cm hair segment was used for mercury analysis [28]. Detailed quality assurance data showed acceptable analyses for both exposure biomarkers. Results in micrograms (μg) may be converted to nanomoles (nmol) by multiplying by 5.0. The study protocol was approved by the Ethical Review Committee for the Faroe Islands and the Institutional Review Board at Harvard School of Public Health. Written informed consent was obtained from all parents, and assent from the adolescents. Further detailed descriptive information about the cohort mercury concentrations and the neuropsychological outcomes used among the participants at age 7 and 14 years are published elsewhere [11,16].

2.1. Outcome examination

We administrated the Neuropsychological Examination System (NES2) Continuous Performance Test (CPT) [24]. For the purpose of these birth cohort studies, animal silhouettes were drawn so that the shaded area covered approximately the same area as the conventional version that uses letters, disregarding the relative size of the real animals they represented. The head was drawn in the same direction for all animals, and the "cat" was selected as the critical stimulus. The participant was instructed to press the joystick key as quickly as s/he could every time the cat appeared on the computer screen, but not for other animals. A total of 100 critical stimuli were presented during a 10-min test at age 14 years. The stimulus duration was 1000 ms, and each stimulus was replaced on the screen instantaneously with the succeeding one. Failure or a slow response outside of the time given to a critical stimulus within the 1000 ms was considered a non-response (omission error), and a response to a stimulus other than the cat was recorded as a false alarm response (commission error). A Hit Reaction Time (HRT) measure was derived when a positive response was recorded within 1000 ms after the appearance of the "cat" on the screen (i.e., in time before the next stimulus). In the default version of the CPT, a mean hit reaction time is determined for true positive responses. As this measure does not take into account the omissions, all no-responses to the cat stimulus were scored as 1000 ms. The HRT average and the median were then calculated, thus preventing any possible distortion of the results due to omissions.

Because motor speed and simple reaction time may affect the HRT results, we also included these functions assessed by the Catsys® equipment (Danish Product Development, 3070 Snekkersten, Denmark) which allows electronic assessment of simple reaction time using joint auditory and visual stimuli, as well as tapping speed.

2.2. Data analyses

While the HRT is measured by CPT, it is employed to assess the speed of information processing in which different neuropsychological functions may be involved depending on time duration. Thus, three stages were defined for the purposes of this study:

1) CPT-HRT1: The first 2 min were considered a training period in which the HRTs were probably influenced by factors such as orientation, learning and habituation. Previous studies eliminated the early trial period of the CPT duration when analyzing the HRT [10,11,16].
2) CPT-HRT2: The middle stage (3–6 min) was presumed to reflect the cognitive processing speed and selective focused attention.
3) CPT-HRT3: The final minutes (7–10 min) were presumed to assess sustained attention as well.

After the first two-minute training period, the remainder of the test duration was split into two parts of equal size, i.e., 4 min each.

Table 1
Correlation coefficients between the outcome variables (CPT-HRT of NES2 Test).

Continuous performance test (CPT) reaction time (HRT) outcomes (average in ms)			
	3–6 min	7–10 min	3–10 min
1–2 min	0.76	0.69	0.75
3–6 min	1.00	0.86	0.96
7–10 min		1.00	0.97
3–10 min			1.00

1–2 min. (CPT-HRT1); 3–6 min. (CPT-HRT2); 7–10 min. (CPT-HRT3); 3–10 min. (CPT-HRT2 + 3).
CPT-RT correlations with Catsys-simple Reaction Time (SRT) and Finger Tapping (FT) were around 0.30.

Table 2
Correlation coefficients between CPT-HRT latencies per minute and the selected determinants for different test durations.

	Selected determinants				
Duration of test (minutes)	Log cord blood Hg conc.	Log blood Hg conc., 14y	Catsys-simple reaction time (SRT)	Finger tapping (FT)	CPT-HRT1
First	0.05	0.07	0.28*	−0.24*	–
Second	0.03	0.05	0.24*	−0.24*	–
Third	0.07*	0.05	0.27*	−0.27*	0.69*
Fourth	0.06	0.03	026*	−0.31*	0.68*
Fifth	0.06	0.08*	0.24*	−0.26*	0.65*
Sixth	0.09*	0.07	0.28*	−0.25*	0.64*
Seventh	0.09*	0.09*	0.27*	−0.28*	0.61*
Eighth	0.07*	0.06	0.28*	−0.29*	0.62*
Ninth	0.07*	0.02	0.27*	−0.27*	0.62*
Tenth (last minute)	0.11*	0.05	0.28*	−0.26*	0.60*

CPT-HRT1 (1–2 min.).
No significant correlations between Log cord blood Hg concentration and CPT commission and omission errors (data not shown).
*P-value<0.05.

Although these cut-offs were arbitrary, they relate to our prior hypotheses about the relative impact of different functional domains. The two last stages (the middle and final) were combined as a fourth outcome (3–10 min) to compare with previous analyses [11].

Regression analyses were used to determine the associations between the outcomes and methylmercury exposure biomarkers, while adjusting for covariates. The covariates selected were the same ones used in the previous study [11]. Thus, the major obligatory confounders were age, school grade and sex. Likewise, both maternal and paternal employment were included, as was the time of the day of the examination (morning/afternoon), the language used (Faroese/Danish), the computer experience (at home/elsewhere), and hand dominance. Maternal Raven scores and residence in town/village were not included as mandatory covariates. The former was not a confounder for this specific outcome, and residence was considered a possible surrogate for methylmercury exposure. Nevertheless, complementary models were reassessed including these two variables. In further analyses, an extended set of covariates included the Catsys scores of the simple reaction time and motor speed, as well as the CPT-HRT during the first 2 min.

Contaminant determinants entered the model after logarithmic transformation of the mercury concentrations, and the mercury regression coefficients were calculated to correspond to the change in the dependent variable associated with a 10-fold increase in methylmercury exposure. Thus, these models assume a linear effect of log transformed mercury concentration on the neuropsychological outcomes. We used generalized additive models to assess whether that assumption was appropriate, as these models do not require linearity assumptions while providing a smooth non-parametric dose–response curve [20]. Since there were a fewer observations of umbilical cord blood concentrations than maternal hair concentrations at parturition, we calculated imputed cord blood concentrations for the missing values ($n=26$) from maternal hair concentrations using the average ratio for paired samples. Similar calculations were performed for the postnatal exposures ($n=80$).

3. Results

All three CPT-HRT outcomes approached a normal distribution. The mean HRT increased through the three time intervals, with CPT-HRT1 showing the lowest mean of 482 ms, followed by CPT-HRT2 at 494 ms and the highest mean of 514 ms for CPT-HRT3. Table 1 shows the correlation coefficients between the CPT-HRT outcomes, all of which were statistically significant. The lowest correlation observed ($r=0.69$) was found between CPT-HRT1 and CPT-HRT3. Lower correlations, although still significant, were found with the Catsys outcomes.

Table 2 describes the correlation coefficients between the CPT-HRT latency averages during each of the 10 min, the cord blood and 14-year

Table 3
CPT-HRT outcomes by socio-demographical covariates of interest.

		Hit reaction time outcomes (average in ms)			
Participant covariates		1–2 min	3–6 min	7–10 min	3–10 min
Sex	Female ($n=433$) (median)	465	480	504	493
	Male ($n=430$) (median)	481*	494*	513	502*
Dominant hand	Right-handed ($n=776$) (median)	471	486	507	496
	Left-handed ($n=82$) (median)	487*	499*	522	507*
Age at testing, years	(Spearman) ($n=863$)	−0.10*	−0.10*	−0.11*	−0.10*
Grade at school	(Spearman) ($n=863$)	−0.06	−0.07*	−0.10*	−0.09*
Performance with glasses	No ($n=744$) (median)	471	488	509	498
	Yes ($n=119$) (median)	482	490	508	498
Computer experience	At home ($n=765$) (median)	472	486	506	496
	Elsewhere only ($n=98$) (median)	478	509*	528*	512*
Computer game experience	No ($n=564$) (median)	574	490	510	500
	Yes ($n=288$) (median)	570	483	505	495
Town	Villages ($n=404$) (median)	478	490	511	500
	Towns ($n=360$) (median)	470	485	506	496
	Denmark ($n=98$) (median)	463	488	500	490
Time of the day tested	Morning ($n=438$) (median)	473	488	508	499
	Afternoon ($n=423$) (median)	472	488	509	497
Tested in language	Faroese ($n=797$) (median)	474	489	510	500
	Danish ($n=66$) (median)	463	475	499	486
Parental covariates					
Mother employed (14 y)	Yes ($n=686$) (median)	471	485	504	495
	No ($n=172$) (median)	486*	496*	517*	507*
Father employed (14 y)	Yes ($n=791$) (median)	472	488	508	497
	No ($n=54$) (median)	476	497	516	502
Maternal IQ, Raven scores	(Pearson) ($n=798$)	−0.11*	−0.11*	−0.10*	−0.11*

1–2 min. (CPT-HRT1); 3–6 min. (CPT-HRT2); 7–10 min. (CPT-HRT3); 3–10 min. (CPT-HRT2 + 3).
*P-value<0.05 for correlation tests or tests of median differences of the outcomes, both, by the covariate variables and their categories.

blood mercury concentrations, as well as the related neuropsychological functions, Catsys Simple Reaction Time (Catsys SRT) and Finger Tapping (FT), and the CPT-HRT1. The cord blood concentration showed better correlations at later latencies, with the highest coefficient during the last minute. The current blood mercury concentration showed less clear correlations. The neuropsychological covariates (Catsys SRT and FT) showed significant correlations that did not change over time. Lastly, the CPT-HRT1 was positively correlated with CPT-HRT latencies from minute 3 to minute 10 observing a slightly but progressive decreasing tendency of the coefficients.

Table 3 describes the CPT-HRT outcomes by covariates of interest other than the methylmercury exposure biomarkers. Male adolescents, participants who were left-handed, had limited computer experience, and whose mothers who were currently unemployed, as well as those who scored lower on the Raven IQ test tended to have increased HRT scores (i.e., higher latencies). In addition, the subjects' age and grade were negatively related to the HRT scores. All three CPT-HRT outcomes showed similar association to the covariates. Indeed, when the CPT-HRT outcomes were separated into each of the 10 min the results were unchanged (data not shown).

Table 4 illustrates the correlation coefficients between the CPT-HRT outcomes and the mercury concentrations at all examinations, i.e., at parturition, 7 years and 14 years. The only statistically significant correlations were in regard to the cord blood and maternal hair concentrations. CPT-HRT3 had the highest positive correlation coefficients. This tendency was not observed for the postnatal methylmercury exposure, where all coefficients were smaller.

Table 5 describes four different multivariate models analyzing the associations between the cord blood mercury concentration and the different CPT-HRT outcomes. The first model (second column) was adjusted by the selected covariates from Table 3. This model showed statistically significant associations between the CPT-HRT outcomes with the exception of CPT-HRT1. An increasing tendency through the different CPT-HRT stages was observed, showing the strongest association in CPT-HRT3. The second model (third column) was additionally adjusted for the Catsys Simple Reaction Time (Catsys-SRT), where the results were materially unchanged including the increasing tendency. The third model (fourth column) included Catsys Finger Tapping (Catsys-FT) instead of Catsys-SRT, again with similar association patterns, which was also noted in the last model (fifth column) that included CPT-HRT1 as a covariate. When we included all the covariates at the same time, the results were again virtually the same (data not shown). Additionally, when medians were used instead of means for the CPT-HRT outcomes, the results were the same for all models, as were they when the CPT-HRT scores were calculated using hits only (data not shown). The same was true when disregarding imputed mercury concentrations (data not shown). When the maternal Raven score and residence were included, the effects were slightly reduced and the statistical significance decreased, though in part due to missing Raven information for 62 of the total cases (data not shown). Finally, using the maternal hair mercury concentration at parturition instead of the cord blood concentration as a determinant showed once again the same association patterns for all four models, but with less strength (data not shown).

Table 6 describes the same regression models as Table 5, but includes the current blood mercury concentration as the exposure variable. These results did not replicate the association pattern apparent for the prenatal exposure. All the associations were weak and did not reach statistical significance; additionally there was no clear tendency between the different CPT-HRT outcomes. When the models were adjusted for the neuropsychological covariates, the coefficients were severely weakened. This also occurred when using the current hair mercury concentration or the methylmercury exposure biomarkers at age 7 years (data not shown).

When repeating the multivariate model without neuropsychological covariates included, the number of omission and commission errors showed no significant associations with the cord blood mercury concentration, but similarly as HRT the omission errors showed stronger associations during the CPT-HRT3 stage (data not shown).

Table 4
Correlation coefficients between the outcome variables and the selected determinants.

	CPT Hit Reaction Time Outcomes (average in ms)			
Pollutant exposures	1–2 min	3–6 min	7–10 min	3–10 min
Log cord blood Hg concentration ($n=840$)	0.04	0.08*	0.10*	0.09*
Log maternal hair Hg concentration, parturition ($n=861$)	0.03	0.07*	0.09*	0.08*
Log blood Hg concentration, 7 y ($n=606$)	0.06	0.06	0.07	0.07
Log hair Hg concentration, 7 y ($n=805$)	0.03	0.04	0.06	0.05
Log blood Hg concentration, 14 y ($n=781$)	0.07	0.06	0.06	0.06
Log hair Hg concentration, 14 y ($n=845$)	0.06	0.06	0.06	0.06

1–2 min (CPT-HRT1); 3–6 min (CPT-HRT2); 7–10 min (CPT-HRT3); 3–10 min (CPT-HRT2 + 3).
*P-value < 0.05.

Table 5
Adjusted effects† of 10-fold increase of cord blood mercury concentration¶ on CPT-HRT outcomes at 14 years of age ($n=834$).

CPT reaction time outcomes (average in ms)	Covariates	Covariates + Catsys-SRT	Covariates + finger tapping	Covariates + CPT-HRT1
	Beta (SE)	Beta (SE)	Beta (SE)	Beta (SE)
1–2 min	3.41 (2.06)*	2.04 (1.97)	2.12 (1.97)	–
3–6 min	6.10 (2.18)***	4.63 (2.08)**	4.58 (2.05)**	3.39 (1.45)**
7–10 min	7.64 (2.39)***	6.03 (2.28)***	6.09 (2.26)***	4.94 (1.74)***
3–10 min	6.87 (2.19)***	5.33 (2.09)**	5.34 (2.06)**	4.17 (1.47)***

1–2 min (CPT-HRT1); 3–6 min (CPT-HRT2); 7–10 min (CPT-HRT3); 3–10 min (CPT-HRT2 + 3).
†Each line represents 4 multivariate linear regression models adjusted for: Age, sex, school grade, maternal and paternal employment, time of day, language, hand dominance and computer experience. Each column indicates additional adjustments.
¶Mercury concentration is log transformed and imputed from maternal hair concentrations during parturition for the missing values ($n=26$). Results did not change without the imputations (data not shown).
An increase in the test parameter denotes a deficit.
*P-value < 0.10; **P-value < 0.05; ***P-value < 0.01.

Table 6
Adjusted effects† of 10-fold increase of current blood mercury concentration¶ on CPT-HRT outcomes at 14 years of age ($n=835$).

CPT reaction time outcomes (average in ms)	Covariates	Covariates + Catsys-SRT	Covariates + finger tapping	Covariates + CPT-HRT1
	Beta (SE)	Beta (SE)	Beta (SE)	Beta (SE)
1–2 min	1.96 (2.06)	1.00 (1.97)	0.69 (1.96)	–
3–6 min	2.30 (2.18)	1.25 (2.08)	0.77 (2.05)	0.74 (1.45)
7–10 min	2.69 (2.39)	1.52 (2.28)	1.13 (2.26)	1.13 (1.75)
3–10 min	2.50 (2.20)	1.39 (2.09)	0.96 (2.07)	0.94 (1.47)

1–2 min (CPT-HRT1); 3–6 min (CPT-HRT2); 7–10 min (CPT-HRT3); 3–10 min (CPT-HRT2 + 3).
†Each line represents 4 multivariate linear regression models adjusted for: Age, sex, school grade, maternal and paternal employment, time of day, language, hand dominance and computer experience. Each column indicates additional adjustments.
¶Mercury concentration is log transformed and imputed from participants hair concentrations at 14 years of age for the missing values ($n=80$). Results did not change without the imputations (data not shown).
An increase in the test parameter denotes a deficit.
*P-value < 0.10; **P-value < 0.05; ***P-value < 0.01.

4. Discussion

The present findings demonstrate that the associations with prenatal exposure to methylmercury depend on the duration of the CPT task. The positive associations reflect an increasing HRT latency, and slower responses are an indication of a cognitive deficit. Even though the three stages were highly inter-correlated, the CPT-HRT1 seemed to represent a learning and habituation period and was less clearly associated with methylmercury exposure. CPT-HRT2 was hypothesized to involve speed processing and selective focused attention and was highly associated with prenatal methylmercury exposure, even after controlling for motor speed, simple reaction time, and CPT-HRT1. Nevertheless, CPT-HRT3, where we believe sustained attention is the dominant demand, showed the strongest associations with prenatal methylmercury exposure in all multivariate models examined. Current mercury concentrations did not show any clear association, and the coefficients approached zero when adjusted for other neuropsychological functions, particularly motor speed.

Previous epidemiological studies have applied CPT as a tool for assessing attention function, but analyzed the outcomes without considering the time from test initiation [1–3,11,16,33]. These studies also did not investigate the plausible involvement of different functional domains in relation to test duration, although some excluded the first minutes as a practicing period [10,16]. In the present study, with cord blood mercury concentration as a determinant, the commission and omission errors showed weak and non-significant associations. Nevertheless, the HRT latencies were the most sensitive CPT outcomes, in regard to methylmercury neurotoxicity [11,16].

The associations between prenatal methylmercury exposures and CPT-HRT were stronger in the last stage, which suggests that the effect might be primarily on sustained attention rather than other functional domains involved in completing the early stages of this task. The current methylmercury exposures, although not statistically significant, showed similar coefficients in all of the CPT-HRT stages, but when adjusted for motor speed the weak coefficients almost disappeared. This association pattern could indicate that current exposure to methylmercury, rather than prenatal, affects other neuropsychological functions, such as motor speed, and thus simple reaction time to a similar extent in each of the three CPT-HRT stages. This hypothesis is indicated by a previous study of the cohort subjects based on an extended battery of neuropsychological tests [11].

Prenatal exposure to methylmercury seems to have a stronger neurotoxic effect that also differs qualitatively from the effects of postnatal exposure [11]. In regard to other neurotoxicant exposures, such as pesticides, tobacco smoke and polychlorinated biphenyls, similar conclusions have been reached [6,18,19,21,22,32]. Overall, the detailed analyses of the 10-minute duration repetitive task extend the conclusions from the previous study [11]. Sustained attention function as indicated by several neuropsychological tests, was one of the most strongly affected domains in regard to prenatal methylmercury exposure [11]. However, sustained attention is a very complex cognitive function in which factors such as motivation and cognitive control play an important role. This neuropsychological function is predominantly supported by activity in the frontal lobes, particularly the cingulate and prefrontal areas [6,11,23,34,35], and the sensitivity of this brain region to early developmental methylmercury exposure therefore deserves attention.

Some test limitations could obscure the findings, for example, visual acuity may play a role due to the CPT test characteristics given that the adolescent is required to watch a computer screen [17]. But if this is the case, the results of the three CPT-HRT stages would be similarly affected, since the task is exactly the same during the entire test period. Moreover, a prior report based on this cohort concluded that prenatal exposure to methylmercury did not affect visual function [17]. Additionally, the computer experience influenced the results of CPT, but all the CPT-HRT outcomes were similarly affected by this factor, and the methylmercury exposure coefficients were unchanged after adjusting for this covariate.

The decision of where to place the cut-offs between the three CPT-HRT stages was arbitrary. However, this determination was made prior to the start of the data analyses and was based on the knowledge and expertise of a neuropsychologist, who also took into account the statistical properties of the new outcomes. In relation to the two-minute first stage, the intention was to be in consonance with the cut-offs made by other epidemiological studies [11,16]. The separation of the next two stages (selective focused attention and sustained attention) was based on the statistical decision that they should have the same time duration (i.e., 4 min), and consequently the same number of trials per stage. In neuropsychological terms, it is impossible to know where the exact limits between the three stages are, but the most important issue for this study is their comparison and to understand the tendencies observed. Quite possibly, the last minute may be the most informative, although it included only 10 stimuli and therefore less robust data.

The results presented here illustrate a novel use of the CPT-HRT latency results. This outcome is sensitive to the neurotoxic effects of environmental insults [11,16]. Using it in a more comprehensive way (i.e., dividing it by the three mental stages) may help to better interpret which neuropsychological function is more involved. Our findings emphasize the use of HRT-CPT stages in other epidemiological studies, especially when study resources and the timing are limited and the administration of long batteries of neuropsychological tests would be impractical.

5. Conclusions

The CPT-HRT latencies reflect different functions, as indicated by the results separated according to the test duration. The fact that the prenatal methylmercury exposure showed different effects in each of the three CPT-HRT outcomes suggests that different underlying neuropsychological functions are activated in each outcome, although the task is the same during the entire test duration. Moreover, the methylmercury effects persisted with similar patterns after adjusting for functions such as simple reaction time and motor speed, thereby suggesting that other neuropsychological functions may be involved. The sustained attention functioning may be particularly susceptible to prenatal methylmercury neurotoxicity because of the stronger effects observed during the final part of test duration (i.e., CPT-HRT 3). Such deficits indicate probable underlying dysfunctions of the frontal lobes. However, this association may be missed if the test results are averaged as a single outcome. Current exposure to methylmercury is less important and seems to reflect mainly a psychomotor dysfunction. Future studies should use similar groupings of CPT-HRT latencies to explore the underlying dysfunctions (without changing the measurement instrument). This way, the neuropsychological interpretation of CPT-HRT results will better relate to sustained attention and other functional domains playing a role in this specific task.

Conflicts of interest

Philippe Grandjean has provided paid expert testimony on mercury toxicology for the U.S. Department of Justice in a legal case concerning environmental pollution from coal-fired power plants. The authors of this paper otherwise have no financial or personal relationship with people or organizations that could inappropriately influence the work submitted.

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