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Association between methylmercury environmental exposure and neurological disorders: A systematic review

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ABSTRACT

The mercury-related central nervous system disorders have been extensively studied on animal models and human beings. However, clinical evidences of which neurological changes are in fact associated with mercury exposure remains controversial. This systematic review (Prospero registration under the number CRD42016041760) aimed to elucidate the association of methylmercury (MeHg) exposure with neurological alteration in populations living in MeHg-endemic risk area. A systematic search was performed according to the Preferred Reporting Items for Systematic Review and Meta-Analysis criteria using available databases PubMed, LILACS, Scopus, Web of Science, The Cochrane Library, OpenGrey and Google Scholar. A search of the following terms: "methylmercury compounds", "organomercury compounds", "neurologic manifestations", "memory disorders", "neurobehavioral manifestations" and "communication disorders" were performed in a systematic way. Studies focusing on MeHg exposure and subsequent neurological alteration on humans (> 13 years) were included. Evaluation of methodological quality and risk of bias as well as the level of evidence was performed. Our results have identified 470 studies and six articles were eligible for systematic review inclusion criteria. The studies suggested alterations related to the psychosensory, motor and coordination system, as well as motor speech, hearing, visual impairment, mood alterations and loss of intelligent quotient. Of all the six studies, two presented a high risk of bias, with methodological problems related to the confounding factors and all studies presented evidence level ranged from very low to low. In this way our results revealed that a definitive demonstration of an association of MeHg and neurological alterations in human beings is still a pending subject. Future studies in this topic should take into consideration more confident and reliable methods to answer this question.

1. Introduction

Methylmercury is a worldwide pollutant with high toxicity [1]. In 2013, the Minamata convention was signed by many countries to provide human health and environmental protection from mercury exposure [2]. Human exposure to MeHg is a world health issue especially on population who has a daily fish-rich diet [3,4]. Pursuing to understand the MeHg-physiologic effects on live organism, many authors have studied the *in vitro* and *in vivo* MeHg-toxicity mechanism [5].

Central Nervous System (CNS) is the primary and the most sensitive target organ for MeHg, leading to neurologic disturbs as visual impairment, ataxia, paresthesia, neurasthenia, hearing loss, dysarthria, neurodegeneration and muscle tremor [6–12]. Those neurological alterations could be associated with MeHg-induced biochemical, cellular and histological damages on several CNS areas as cerebellum, hippocampus, amygdala, hypothalamus, pre frontal cortex, thalamus and visual, hearing, sensory and motor cortex [13–16]. However, it is important to notice that several studies on this topic are performed on

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animal and culture models. Even though several clinical reports have been reported in humans, the epidemiologic studies that show the relationship of neurological signs and MeHg dose response have been considered to be unclear or imprecise due to several issues, such as: weak methodologies, lack of MeHg or inorganic mercury dosage, control groups and inclusion of confounding factors. In this way, mixed conclusions have been presented regarding the MeHg association with CNS disorders in humans [17,18]. Those differences could be explained by erroneous experimental designs and biases, as age, pre-established disease, and social-economic characteristic of study population.

In this way, our systematic review aimed to answer the following question "Is there an association with MeHg exposure and neurologic alteration on humans?". To address this question we have included studies that show humans with clinical signs of neurological damage and who live at exposed MeHg-related areas. Answering this question, we seek to bring clarification with clinical evidences of MeHg toxicity on humans and also to propose more accurate methodology design to address this question.

2. Material and methods

2.1. Protocol and registration

This systematic review was delineated according to *Preferred Reporting of Systematic Review and Meta-analyses* (PRISMA) [19] and registered on PROSPERO under the number CRD42016041760.

2.2. Search strategy

We performed a systematic search on literature in the following electronic database: PubMed, MEDLINE, Latin American and Caribbean Health Sciences Literature database (LILACS), Scopus, Web of Science and The Cochrane Library. Grey literature (OpenGrey) and Google Scholar were also searched. The searches were performed between July/2016 and December/2017. There was no language restriction on search. MESH and free terms were combined according to syntax rule of each database. We searched for terms related with MeHg exposure and all types of neurological alteration on humans. The neurological disorders terms search were related to neurobehavioral alterations, memory, communicative and cognitive disorders, motor and visual dysfunctions. All MESH terms were showed in Appendix A and were combined using *Boolean* operators (OR, AND).

Studies that were searched in more than one database were counted only once. After selection of studies it was done a hand search on the reference list of each paper in order to include additional studies. In addition, alerts for search strategies were created on each database.

2.3. Selection and eligibility criteria

Duplicated studies were identified using EndNote® (version X7, Thomson Reuters). The selection process were carried out independently by two reviewers (BP and LKRL) based on the acrostic PECO: **(P- p**opulation) Humans; (**E- e**xposure) Methylmecury exposure**; (C- c**omparison) Reference area or population**; (O- o**utcome). The clinical evidence for neurological impairment in humans exposed to MeHg. Null hypothesis tested on this systematic review was that there is no association between MeHg human exposure and the development of neurological alterations.

The studies were selected independently by two reviewers (BP and LKRL), filtering primarily by title and abstract and lastly by the full text reading. A third reviewer (RRL) was consulted when there was disagreement about studies inclusion on this present review. To the final studies, the inclusion criteria were as follows: studies performed on humans < 13 years old, with MeHg/tHg dosage at least in one type of tissue (hair and/or blood) and at least one neurological assay. We excluded case series, descriptive studies, opinions articles, guidelines and editorials, animal and *in vitro* studies.

2.4. Data extraction

Data extraction was carried out by BP and LKRL independently. It was taken into consideration information related to local and year of edition, experimental and reference population, source of samples, characteristics of participants (sample size, age and sex); evaluation method (MeHg/tHg dosage and neurological assays), results and statistical analysis. A third reviewer was also consulted in case of disagreement.

2.5. Risk of bias analysis

The evaluation of methodological quality and risk of bias were performed according to protocol described by Fowkes and Fulton's [20]. This protocol is based on questions about study design as well as sample, characteristic of control group, method and quality of results, drops out or loss of samples, integrity and distorted influence. Each question was answered according to the following code: (0) – no problems; (+) – minor problem; (++) – major problem; (NA) – not applicable. The assessment for each item was standardized by authors and was showed in Appendix B. Three summary questions about bias, confusion and probability of chance were answered after the complete analyze of methods and results found on each select study. To those questions it was attributed the answer YES or NO according to two researches (BP and LKRL) independently. If the answer to three of those questions was NO the study was considered reliable and with low risk of bias.

2.6. Level of evidence

A summary of the overall strength of evidence was presented using "Grading of recommendations, assessment, development and evaluation" (GRADE) tool [21]. Included studies were evaluated according to their design, study quality, consistency, and directness.

3. Results

To perform this systematic review we first identified the properly MESH terms on the National center for Biotechnology Information (NCBI). Terms related to "methylmercury compounds", "organomercury compounds", "neurologic Manifestations", "memory disorders", "neurobehavioral manifestations" and "communication disorders" were searched. All terms are shown on Appendix A. After this search, a total of 714 titles/abstracts were identified. An additional study was also included after the manual search (Fig. 1). After search for duplicate titles/abstracts, 245 studies were eliminated remaining 470 studies.

All 470 titles/abstracts were analyzed being 31 elected for full reading, according to the criteria of inclusion in this systematic review (see section 2.3.Selection and eligibility criteria). Among those 31 articles, 12 were excluded because it was performed in children under 13 years. Twelve studies were eliminated due to the absence of control or reference group and 1 study was excluded for being a statistical computational model. Only six studies fit the criteria and were included in the final analysis. Table 1 show the summary and characteristics of the six studies selected for quantitative analysis.

3.1. Characteristics of included studies

The six studies included in this systematic review were performed by universities or hospitals. The population included in the six studies was composed by adult humans (> 13 years old) living in areas of environmental exposure to methylmercury, with fish as the main source of protein in the diet. Four studies [22–25] were performed on Japan, one in Canada [18] and one in Brazilian Amazon [26]. Among them,

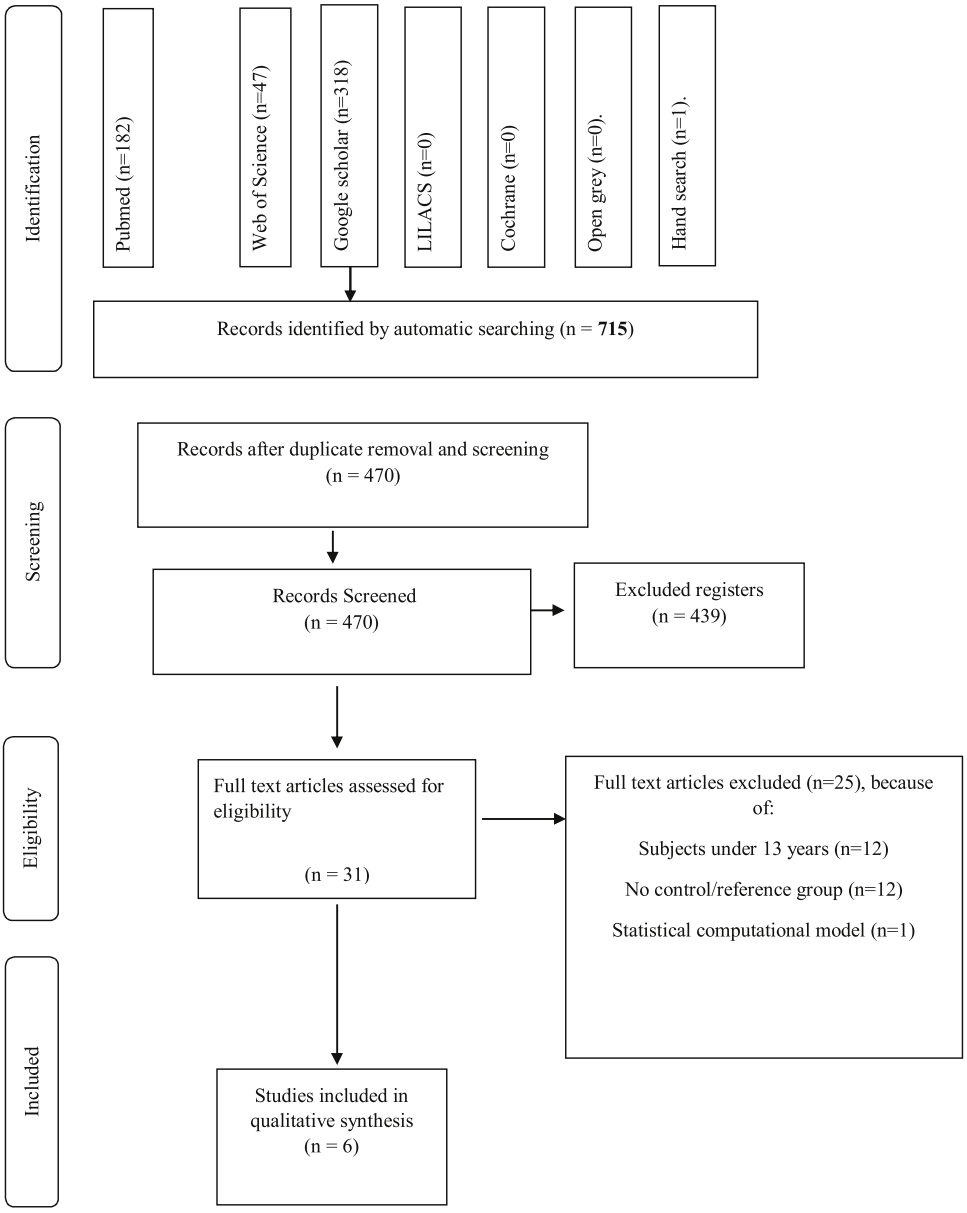

Fig. 1. Flowchart diagram of literature search according to PRISMA guidelines.

two studies were classified as cross-sectional [24,25] and the remaining studies were classified as case-control. The total number of subjects in the six studies is showed in Table 1.

Only two have studies [23,26] quantified mercury levels in hair of participants. Khoury et al. [26] analyzed 108 exposed subjects (with mean mercury content of 8.8 ± 8.53 ppm) of the Tapajós river basin – a region under the influence of gold-mining activity - and 49 control subjects (with mean mercury content of 0.73 ± 0.59 ppm) of Tocantins river basin with no history of gold-mining activity influence. Although Takaoka et al. [23] indicated in the study design that they assessed exposed and non-exposed subjects, this information was not precise. According to the description, they distributed the participants in 59 exposed subjects and 28 non-exposed subjects; however, mercury levels (ppm) were similar in both groups, with no significant difference (2.8 ± 1.5 ppm in control group *vs.* 2.4 ± 1.9 ppm in exposed subjects).

Although not all studies quantified the dose of mercury exposure in the participants, all of them adopted reference areas as control population in order to associate mercury exposure and neurological disorders. They analyzed population under similar demographic characteristic but with different consumption of Hg-contaminated food. For example, in the study performed by Ninomiya et al. [22] the residents of two villages were included on the study. According to the authors, people from Ooura village consumed contaminated fish of Shiranui Sea while people from Ichiburi consumed non-polluted fish from Huganada Sea.

Yorifuji et al. [24,25] on the other hand, adopted three areas with different exposure patterns. The reference area was “Ariake area” and was reported to have low history of contaminated fish; the “Goshonoura area” was referred as mild-contaminated area, as the median level of Hg was around 21.5 ppm; while “Minamata area” was referred as high-contaminated area with a median Hg level of 30 ppm. In the study performed by Takaoka et al. [18], a Canadian population was compared to a Japanese control group from Kumanoto city and with an exposed Japanese group from Minamata area.

The populations comprised both male and female sexes being distributed in a similar way in five studies [18,22–25] and with predominance of female participants in Khoury et al. [26]. The age range was from 13 to 85 years, being excluded children under 13 to avoid distortions during psychosensory tests. Neurologic assays were

Table 1
Characteristic of samples and data of included studies.

Author, year	Sample				Methods of evaluation			
	source of sample	Sample size	Age	sex	Hg Dosage		Neurological assay	
					Sample	Method	Fine-surface-texture discrimination	Two-point discrimination
Ninomiya et al. [22]	Japan	n = 263	> 20 years	Male = 48.5% (Ooura) and 46.5% (Ichiburi)	No information	No information	–	–
Takaoka et al. [23]	Minamata Kyoritsu Hospital	n = 87	> 20 years	Male = 41.37% Female = 50.57%	Hair	cold vapor atomic absorption	Grit value: 30, 12, 9, 5, 3, and 1 μm Standard stimulus: 3 μm. Time: 15s Interstimulus interval: 5s Number of trials: 60 Standard stimulus: 3 μm. Time: 15s Interstimulus interval: 5s Number of trials: 60	–
				Female = 50.57%				
Yorifuji et al. [24]	Japan	n = 3038	> 10 year	Male = 44.89% Female = 55.10%	No information	No information	–	–
Yorifuji et al. [25]	Japan	n = 3038	> 10	Male = 44.89% Female = 55.10%	Unevaluated	Unevaluated	–	–
Takaoka et al. [18]	Ontario, Canada	n = 80	> 15	Canadian Male = 4625%; Japan Male = 4167% Canadian Female = 5375%; Japanese Female = 5833%	No information	No information	–	"Yes/No" method and "2-alternative"
Khoury et al. [26]	São Luís do Tapajós and Barreiras, Tapajós-Brazil. Furo do Maracujá, Tocantis-Brazil	n = 157	> 13	male = 22.2% Female = 77.8% male = 36.7% Female = 63.3	Hair	cold vapor	–	Lower lip; right and left index fingers

Author, year	Methods of evaluation								
	Neurological assay								
	Stereognosis	Paresthesia/ Hypoesthesia	vibratory	Ataxia	Tremor	Normal/ Tandem gait	Finger nose	Reflex	Balancing one-foot
Ninomiya et al. [22]	–	Pinprick and light touch	–	Pronating and supinating the tapping hand, finger nose and heel-shin test and gait	–	–	–	–	–
Takaoka et al. [23]	–	–	–	Diadokokinesis, finger-nose test; tandem gait or stand on one foot;	–		–	–	

(continued on next page)

Table 1 (*continued*)

Author, year	Methods of evaluation								
	Neurological assay								
	Stereognosis	Paresthesia/ Hypoesthesia	vibratory	Ataxia	Tremor	Normal/ Tandem gait	Finger nose	Reflex	Balancing one-foot
Yorifuji et al. [24]	–	whole body, extremities, and perioral area	–	Ataxia including ataxic gait, adiadochokinesis, finger-nose test,	No information	–	ataxic gait, adiadochokinesis, finger-nose test,	pathologic reflexes including Hoffman reflex, Babinski reflex,	–
Yorifuji et al. [25]	–	–	–	–	–	–	–	–	–
Takaoka et al. [18]	–	–	128 Hz tuning fork.	–	Postural hand	–	–	–	–
Khoury et al. [26]	Semmes Weinstein	–	upper region of the	–	–	–	–	–	–

Author, year	Methods of evaluation						Results	Statistical analysis
	Neurological assay							
	Visual test	Nystagmus	Hearing test	Dysarthria	Mood and behavior dysfunction	Impairment of intelligence (QI)		
Ninomiya et al. [22]	confrontation	–	Tuning fork of 512Hz and a ticking watch	Slurred speech	–	–	People living in Ooura village showed a significantly higher frequency of neurological signs characteristic of methylmercury poisoning, as hypoesthesia, ataxia, impairment of hearing, visual change and dysarthria than people living in a non-polluted area (Ichiburi). Hypoesthesia showed the highest frequency of change.	Statics comparison was made by a X^2 test
Takaoka et al. [23]	–	–	–		–	–	Exposed subjects with sensory findings (EXP + S) had a higher sensory disturbance (weber fraction = 2.11) when compared to control group (WF = 0.91), whereas exposed subject without numbness and neurological finds showed a mild disturbance of fine-surface-texture discrimination (WF = 1.63)	All data were analyzed by t-Test. A z score were performed to show the difference threshold between the amount of stimulus change in sensation recognition. A linear regression of the stimulus with z score were performed to analyses the MeHg correlation with neurological outcomes

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Table 1 (*continued*)

Author, year	Methods of evaluation						Results	Statistical analysis
	Neurological assay							
	Visual test	Nystagmus	Hearing test	Dysarthria	Mood and behavior dysfunction	Impairment of intelligence (QI)		
Yorifuji et al. [24]	Constriction of visual field	No information	hearing difficulties	–	–	–	Tree villages were evaluated. They were grouped as Low exposure, Medium exposure and High-exposure village. Results showed that population in high-exposure villages manifested neurological signs more frequently. The highest prevalence was for paresthesia around the mouth.	The prevalence of neurologic signs in relation to exposure were estimated with a logistic regression model, adjusted for age and sex.
Yorifuji et al. [25]	–	–	–	–	apathetic, hypobulic, perseverative, euphoric, impatient,	lack of initiative, slow movement or speech, memory disturbance,	PORs for impairment of intelligence and	Authors have
Takaoka et al. [18]	–	–	–	–	–	–	The two groups of Canadian people showed prevalent complaints and significant sensory measurement compared to Japanese control group. Neurological findings and quantitative sensory measurements	The prevalence comparison was tested by x^2. When results were compared by average. *T-test* were used.
Khoury et al. [26]	–	–	–	–	–	–	Hg hair samples ranged -exposed	Student's t-test with Bonferroni multiple-significance-test correction. To evaluate the dependence the psychophysical parameters on the mercury concentration, we investigated the Pearson product-

performed in all the six studies in order to assess the commitment of visual, hearing, cognitive, motor and sensorial behavior associated with MeHg exposure.

In the study performed by Ninomiya et al. [22] the authors assessed motor (coordinated movements), visual (confrontation), hearing and sensory (hypoesthesia and paresthesia) endpoints. Takaoka et al. [23] used the single psychosensory test "Fine-surface-texture discrimination". Yorifuji et al. [24] performed motor (tremor, coordinated movements and reflex), visual (nystagmus and constriction of visual field), hearing and sensory alterations (hypoesthesia and paresthesia). In the study performed by Yorifuji et al. [25] only mood and behavior dysfunction and IQ test were performed. Khoury et al. [26] performed sensory tests (two-point discrimination, stereognosis and vibratory sensations). The description and characteristics of each neurological assessment are shown in Table 1. All six studies aimed to show the association between MeHg exposure and neurological alteration in population living in methylmercury-contaminated areas.

The most frequent neurological alterations showed in exposed participants of the six studies were sensory disturbance [18,22–24,26] followed by motor and coordination impairment [18,22,24], motor speech [18,22,24], hearing impairment [18,22], visual changes [18,22,23], impairment of intelligence and mood and behavior dysfunction [25].

3.2. Risk of bias

For the quality of the assessment, all six studies were classified according to the risk of bias, confounding factors and probability of chance (Appendix C). The following minor (+) and major (++) problems were identified: "sampling methods", "size of samples", "entry criteria/exclusion", "matching/randomization", "comparable characteristics", "blindness", "quality control", "confounding factor" and "distortion reduced by analysis".

None of the six studies have showed sampling methods as sample calculation (++). The study performed by Yorifuji et al. [25] was the only one to mention the representativeness of the sample size (0) while the five others failed in this topic (+). In addition, in the study of Takaoka et al. [23], besides the lack of calculation and representativeness, sample size was lower than 50 participants, being attributed a major problem (++). In the "entry criteria/exclusion" topic, a minor problem (+) was attributed to the study of Yorifuji et al. [24] due to the inclusion of children under 13 (they only excluded children < 10 years old). Taking into account the criteria "matching/randomization", a minor problem (+) was attributed to both Khoury et al. [26] and Takaoka et al. [18]. Takaoka et al. [18] were the only ones to compare populations from different countries. The use of Canadian and Japanese populations for comparing the prevalence of neurological alterations was considered a minor problem (+).

In the "blindness" topic, Takaoka et al. [18] was the only one to mention that the neurological assay was under blindness (0) while the other five studies did not mention or did not performed the blindness (++). However, the authors did not mention the professional who had performed the neurological assay and for this reason was applied to a major problem to the "quality control" (++) while de five other had no problems on this topic (0). In the "confounding factors" the study performed by Ninomiya et al. [22] was attributed to a minor problem (+). The authors did not performed comparison between subjects on the same age group. In the study performed by Takaoka et al. [18] it was applied a major problem (++). In this study were included subjects with and without diseases such as diabetes, stroke, psychiatric diseases, *etc.* This study was also applied to a major problem in the "distortion reduced by analysis" as the inclusion of subjects with neurological-related diseases could lead to misinterpretation of data in the association of MeHg exposure and neurological diseases. Based on these results, only Ninomiya et al. [22] and Takaoka et al. [18] were attributed with YES into all three summary questions. In this way, both studies had their methodological quality compromised and may not be considered soundness.

3.3. Level of evidence

Among the included studies, the prevalence of the evaluated neurological disorders varied from 10.2 to 37.7%, according to each type (Table 2). We did not perform the level of evidence of sensory alterations due to difficulty to group the data provided by included studies. The hearing impairment was the most prevalent disorder reported on the included articles. The certainty among the prevalence of ataxia, hearing impairment, visual constriction, Dysarthria and Postural tremors varied from very low to low. The high risks of bias reported in two articles [18,22] as well as the observational nature of the Studies have contributed to these results.

4. Discussion

Although the relationship between methylmercury exposure and neurological alterations is well established in both *in vivo* and *in vitro* studies, a truly translational knowledge requires a confirmation in humans. Though only controlled interventions are able to demonstrate a causal relationship in humans, demonstrating an association between the two factors (MeHg exposure and neurological disorders in humans) is the prerequisite for the existence of the causal relationship (if no association exists, no cause-effect relation is possible). So, this systematic review aimed to find unquestionable evidences on epidemiological studies with humans that definitively demonstrate an association between MeHg environmental exposure and neurological disorders.

After a critical analysis, five out of six studies showed that the most prevalence alterations were related to psychosensory system, as showed by pain, sensory threshold and touch endpoints, while four out of six showed that motor and coordination alterations were associated with MeHg exposure [18,22–24]. The studies also showed motor speech and visual impairment [18,22,23]; hearing loss [18,22], mood alterations and loss of intelligent quotient [25]. These neurological signs were evaluated for the level of evidence through GRADE, which ranged from very low to low. To our knowledge, this is the first systematic review to perform quality assessment on this topic.

Our results followed a based search on evidences, making use of critical search methods, analysis and summary of the main findings in a systematic way by two reviewers independently [27,28]. Thus, this systematic review aimed to assist professionals in the area, summarizing clinical evidences of neurological alterations in populations living in regions contaminated with methylmercury and indicating differences and similarities on studies that may have the same experimental question, to give perspective on futures investigation.

Inclusion/exclusion criteria were determined according to the relevance to our initial question. Studies with children fewer than 13 were eliminated and only studies with adolescent, adult and aged subjects were included. We also used as inclusion criteria studies that presented a reference group – subjects who lived in regions that was not related to MeHg exposure and who had similar socioeconomic characteristics to the study group. This analysis of paired group is able to reduce the chances of wrong interpretations due to group variability [29]. Regarding to MeHg exposure levels, despite that none of studies have measured the total MeHg on study subjects (only total Hg were measured), all studies selected in this review included populations environmentally exposed through diet (fish). By this *via* of exposure, 70–95% of the total mercury content is usually as methylmercury [30–33]. So, to find an association between neurological disorders and total mercury content in these populations is equivalent to find the same association with MeHg exposure (see Appendix A). In this way, we have included studies that adopted residential area as an indicator of exposure to MeHg, since most studies did not include MeHg or Hg

Table 2
Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) instrument.

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Methylmercury poisoning	Control	Relative (95% CI)	Absolute (95% CI)		
Prevalence of Ataxia												
3	observational studies	serious [a]	not serious	not serious	not serious	dose response gradient	573/2457 (23.3%)	68/972 (7.0%)	not estimable		⨁⨁◯◯LOW	CRITICAL
Prevalence of Hearing impairments												
2	observational studies	very serious [b]	not serious	not serious	not serious	dose response gradient	109/289 (37.7%)	23/142 (16.2%)	not estimable		⨁◯◯◯ VERY LOW	CRITICAL
Prevalence of visual constriction												
3	observational studies	serious [b]	not serious	not serious	not serious	dose response gradient	112/342 (32.7%)	1/306 (0.3%)	not estimable		⨁⨁◯◯ LOW	CRITICAL
Prevalence of Dysarthria												
3	observational studies	serious [b]	not serious	not serious	not serious	dose response gradient	319/2572 (12.4%)	20/1061 (1.9%)	not estimable		⨁⨁◯◯ LOW	CRITICAL
Prevalence of Postural Tremors												
2	observational studies	very serious [b]	not serious	not serious	not serious	dose response gradient	249/2451 (10.2%)	30/919 (3.3%)	not estimable		⨁◯◯◯ VERY LOW	CRITICAL

dosage. However, it is important to notice that the most reliable way to confirm the degree of exposure is by biological monitoring of hair and blood samples dosage [34,35]. Only two studies [23,26] included in this review showed Hg hair dosage, a precise and reliable method to the analysis of populations exposed to MeHg after fish consumption [35].

The Hg-levels monitoring in populations susceptible to exposure is an important tool to understand how MeHg is harmful to living beings. This concern has got attention since the Minamata accident, the first MeHg outbreak to be recognized for serious damage to human health [36,37] and since then several studies have tried to understand how MeHg exposure could impair neurological functions in human beings [38–43]. The Minamata disease was recognized in the late 50′s but clinical evidence was only proven decade's later [44,45]. A study from patients certified with Minamata disease suggested that the major neurological findings were sensory and coordination impairment, constriction of visual field and hearing loss [46]. The latter outbreak was an acute exposure to levels above 50 ppm in hair. Interestingly, the six studies selected in this revision included populations exposed to lower levels of mercury (up to 30 ppm) that could influence in the prevalence of the detected symptoms of neurological alterations. Several studies have shown that once Hg gets to the bloodstream it is widely distributed in several organs as kidney, liver, bone and brain [47–50].In the brain, MeHg induce cytotoxicity since it is able to change the cell homeostatic balance, induce cellular death and alterations on cell proliferation/differentiation [51–53]. The main mechanism by which MeHg exerts its toxic effect seems to be related to biochemical changes on important enzymatic systems as glutathione, superoxide dismutase, cytochrome oxidase and membrane proteins that could change cellular structure and function after MeHg exposure, as well as by inhibition of macromolecules synthesis as DNA, RNA and proteins [54–57]. The oxidative stress and increasing on intracellular Ca^{+} could lead to unbalance on neurotransmitters production, release and recycling [58–60]. Several authors have shown that MeHg has influence on the glutamate homeostasis, in a phenomenon called cytotoxicity [61,62]. Although the selected studies in this present review do not investigate these mechanisms undelying MeHg toxicity, literature search pointed them as those studies that are already been associated with MeHg neurotoxicity.

In the present systematic review, five out of six studies included have performed at least one experimental parameter to study the principal neurological finding and only one [25] had evaluated mood and intelligence parameters. Among these parameters only three sensory and two motor endpoints were analyzed by more than one author (See Table 1), which makes the comparison between observed results hard to follow. For example, in the sensory analysis Takaoka et al. [18] and Khoury et al. [26] performed "Two point discrimination" and "vibration sense". In both studies the authors demonstrated that subjects suspected to be exposed showed worse test scores when compared to those of the reference subjects. However, when Khoury et al. [26] performed a linear correlation with the Hg levels in the hair of participants no significant association was founded. On the other hand, Khoury et al. [26] showed a positive linear correlation between "tactile sense" alterations observed and Hg hair dosage, but none of the other studies have evaluated this sensory endpoint. The same problem was observed about motor, visual and hearing analysis. Ataxia was evaluated by three authors while visual constriction and hearing impairment was evaluated by two authors. In all studies both neurological endpoints were suggested to be associated with the degree of MeHg exposure since the authors made analysis in populations that live in low, medium and high exposure area.

The difference of the analysis showed in the selected studies could be explained by the absence of standard methodology in the MeHg-induced neurological alteration studies. After the checklist analysis, we identified methodological problems such as the absence of adequate description to reduce bias in sampling methods, sample size, entry criteria/exclusion, matching/randomization, blindness, quality control,

confounding factors and distortion reduced by analysis (Appendix C). None of studies have included sample calculation or indicate the representativeness of population. The absence of information could lead to data misinterpretation due to the non-attendance of samples representativeness and methodological validity compromise [63,64]. In fact, it is well know that in case-control studies the sample calculation based on the size of population is an important component to study experimental design and should be used in all studies performed in human beings [65]. The same was observed about the sample size. Only the study performed by Yorifuji et al. [25] has mentioned the percentage of population that had agreed to the investigation.

Another methodological problem refers to group matching by the number of subjects (n). Two of the included studies [18,26] showed differences on the number of subjects between MeHg and reference subjects group. Although the authors provided information about sample randomization, the adequate matching between sample size of control and study group was not used. The matching group is an important toll in case-control studies since could be able to reduce the confounding variables or distribute them in a more similar way between groups [66]. Another important validation parameter between the study groups is the topic “comparable characteristic”. Five out of six studies included in this review have evaluated population with the same socioeconomic, lifestyle and occupations characteristic. Only the study performed by Takaoka et al. [18] did not meet this criterion.

Concerning to the quality assessment of distorting influence, although all the six studies have excluded subjects with any disease that could be associated with neurological alteration, our analysis showed that the major methodological problem is on the confounding factors. One of the main problems was related to the difficulty to access and analysis on MeHg-exposed populations. In Brazilian Amazon, for example, subjects exposed to MeHg usually lives in remote/isolated regions with low or no technical/scientific support to data acquisition and analysis [31–33]. The same is observed around the world. In fact, all the six articles included in this review were based on vulnerable population as riverside and people living in small islands, that has low or none access of health care. This bias could lead to an increase of confound factors being able to erroneously misinterpretation of data about the association of neurological outcomes and MeHg exposure.

Another important bias was the interference of aging. The analysis taking into consideration participant’s age seems to have been extremely important to the relevance of found data. Older age, for example, may be responsible for a higher accumulation of mercury in the human body because the kidneys, the main organs responsible for mercury removal, work worse. Additionally, the neurological functions are influenced by aging and the analysis based on different age group represents an important way to avoid bias [67,68]. This becomes more relevant when we took into consideration the bioaccumulative characteristic of MeHg over time [69–71]. Populations chronically exposed to MeHg shows high levels of Hg on the CNS [31–33]. Thus the MeHg-induced neurological changes in the elderly could be easily confused with the decrease of neurological functions that are often associated with the normal processing of aging [72]. Among the included studies, Ninomiya et al. [22] did not performed analysis by age range while in the study proposed by Takaoka et al. [18] age stratification was indicated only in the MeHg-exposed group with no information about the age of the reference group. It is important to notice that the definition of age groups was not consistent among the included studies, which makes difficult the comparison between the observed results. According to the Medical Subject Headings (MeSH) on the NCBI database the definition of age group is as follows: adolescent (13–18 years of age), adult (19–44 years of age), middle aged (45–64 years of age) and aged (65–79 years of age). In this way, we suggest that next studies in the MeHg-related neurological alteration make use of this standard classification to correctly address the progression of neurological MeHg-related diseases.

Another potential problem observed in the selected articles is the choice of statistical test to show the association between neurological outcome and Hg levels. Four [18,22,23,26] out of six studies have only evaluated the association trough a bivariate statistical analysis. The problem to use this type of analysis is that only two variables are taken into account leaving out other interference factor. On the other hand, two studies [24,25] have performed a multivariate analysis as a regression model adjusted by age and sex. The multivariate regression tests are considered the most adequate to demonstrate associations such as the neurological diseases and Hg exposure. In this way, the statistical analysis is extremely important to correctly address the association of Hg levels and neurological outcomes. In fact, it is appropriate the evaluation of additional characteristics related to population, exposition and the outcome in clinical trials to improve the validity and to reduce potential bias in the study [20].

All methodological problems cited above were confirmed when we evaluated the GRADE among the included studies (See Table 2). A very low to low level of evidence was verified with a critical risk of bias, mainly because the observational study design starts analysis already in a low score. Since the present study is a systematic review of prevalence data, the inclusion of interventional studies was not indicated. The absence of Hg levels monitoring and more strong methodologies suggests the need of more well-designed studies in order to get more reliable answers to the question investigated in this review.

In this way, studies that aimed to show if MeHg could lead to or intensify neurological deficits on mercury exposed population should take into consideration the appropriate study design, seeking to minimize the confounding factors highlighted here in this review. We also emphasize that studies must perform sample calculation based on representativeness of each specific population, choose reference subjects that are similar to the study group with Hg levels monitoring in both groups, besides suggest that all analysis are performed in subgroups according to a standardized age group.

5. Conclusion

This systematic review showed that the methodological problems make week the conclusions on clinical evidences that mercury could impair neurological function on humans living in exposure areas. The most problematic confounder influence during this analysis was the data analysis and age related symptoms. This limitation may have been critical to results found and future studies on this topic should pay more attention on that. This systematic review shows the importance in accurate methodological design and brings the need for further studies to elucidate human CNS disorders related to MeHg.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jtemb.2018.12.001.

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